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(54) PHOSPHORYLATED WAXY POTATO STARCH

PHOSPHORYLIERTE WACHSKARTOFFELSTÄRKE

AMIDON DE POMME DE TERRE GLUTINEUX PHOSPHORYLE

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Remarks:

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Description

[0001] The present invention relates to potato starches having an amylose content of less than 10% by weight, a phosphate content in the C6 position of between 35 and 100 nmol of phosphate per milligram of starch and an increased content of side chains having a DP of from 12 to 19 as compared with the potato starch from corresponding wild-type potato plants.

[0002] In view of the increasing importance which is currently being attached to plant components as renewable sources of raw material, one of the tasks of biotechnological research is to endeavor to adapt these plant raw materials to the requirements of the processing industry. In addition to this, it is necessary to achieve a great diversity of substances in order to enable renewable raw materials to be used in as many areas of employment as possible.

[0003] While the polysaccharide starch is composed of chemically uniform basic units, i.e. the glucose molecules, it is a complex mixture of different molecular forms which exhibit differences with regard to the degree of polymerization and branching and consequently differ greatly from each other in their physicochemical properties. A distinction is made between amylose starch, an essentially unbranched polymer composed of alpha-1,4-glycosidically linked glucose units, and amylopectin starch, a branched polymer in which the branches are formed as a result of the appearance of additional alpha-1,6-glycosidic linkages. Another important difference between amylose and amylopectin lies in their molecular weights. While amylose, depending on the origin of the starch, has a molecular weight of 5×10^5 - 10^6 Da, the molecular weight of amylopectin is between 10^7 and 10^8 Da. The two macromolecules can be differentiated by their molecular weight and their different physicochemical properties, something which can most readily be visualized by their different iodine-binding properties.

[0004] Amylose was regarded for a long time as being a linear polymer which consisted of alpha-1,4-glycosidically linked alpha-D-glucose monomers. However, more recent studies have demonstrated the presence of a small proportion of alpha-1,6-glycosidic branching points (approx. 0.1 %) (Hizukuri and Takagi, Carbohydr. Res. 134 (1984), 1-10; Takeda et al., Carbohydr. Res. 132, (1984), 83-92).

[0005] Amylopectin constitutes a complex mixture composed of glucose chains which are branched differently. Amylopectin is more strongly branched than amylose. Side chains are linked by way of α-1,6-glycosidic bonds to the main chain, which is composed of α-1,4-glycosidically linked α-D-glucose monomers. According to textbook data (Voet and Voet, Biochemistry, John Wiley & Sons, 1990), the α-1,6 branches occur every 24 to 30 glucose residues on average. This corresponds to a degree of branching of approx. 3%-4%. The data with regard to the degree of branching are variable and depend on the origin (e.g. plant species, plant variety, etc.) of the given starch. In typical plants which are used for industrial starch production, e.g. corn, wheat or potato, approx. 20%-30% of the synthesized starch is composed of amylose starch and approx. 70%-80% is composed of amylopectin starch.

[0006] The functional properties, such as the solubility, the retrogradation behavior, the ability to bind water, the film-forming properties, the viscosity, the pasting properties, the freeze/thaw stability, the acid stability, the gel strength and the starch grain size of starches are influenced, inter alia, by the amylose/amylopectin ratio, the molecular weight, the pattern of side chain distribution of the amylopectin, the content of ions, the content of lipid and protein, the mean starch grain size, the starch grain morphology, etc. The functional properties of starch are also influenced by the content of phosphate, in the starch. In this connection, a distinction is made between phosphate which is covalently bonded in the form of monoesters to the glucose molecules of the starch (termed starch phosphate below) and phosphate in the form of phospholipids which are associated with the starch.

[0007] The content of starch phosphate varies in dependence on the plant type. Thus, for example, certain corn mutants synthesize a starch having an elevated content of starch phosphate (waxy corn 0.002% and high-amylose corn 0.013%) whereas conventional corn types only exhibit traces of starch phosphate. Small quantities of starch phosphate are also found in wheat (0.001%) whereas it has not been possible to detect any starch phosphate in oats and sorghum. Relatively large quantities of starch phosphate have thus far been detected in tuber or root storage starch, for example tapioca (0.008%), sweet potato (0.011%), arrowroot (0.021 %) or potato (0.089%). The percentage values of the starch phosphate content which have been cited above in each case relate to the dry weight of the starch and were determined by Jane et al. (1996, Cereal Foods World 41 (11), 827-832). Starch phosphate can be present in the form of monoesters at the C2, C3 or C6 position in the polymerized glucose monomers (Takeda and Hizukuri, 1971, Starch/Stärke 23, 267-272). In general, from about 30% to 40% of the covalently bound starch phosphate groups are located in the C3 position, and from about 60% to 70% are located in the C6 position, in the glucose monomers (Blennow et al., 2000, Int. J. of Biological Macromolecules 27, 211-218).

[0008] Potato amylopectin starches, i.e. starches having an amylopectin content of more than 90% and an amylose content of less than 10%, can be obtained from potato plants in which the activity of the starch granule-bound starch synthase GBSSI ("Granule-Bound Starch Synthase I") is reduced (Shure et al., 1983, Cell 35, 225-233; Hovenkamp-Hermelink et al., 1987, Theoretical and Applied Genetics 75, 217-221; Visser et al., 1991, Mol. Gen. Genet. 225, 289-296). GBSSI is involved in the formation of amylose. Inhibition of the GBSSI activity leads to the synthesis of starch which is almost exclusively composed of amylopectin. The corresponding GBSSI gene in maize is known under the name "waxy".

Amylopectin starches are also termed waxy starches.

[0009] Plants in which the activity of soluble starch synthase III (SSIII) is reduced have also been described (Abel et al., 1996, The Plant Journal 10(6), 981-991; Lloyd et al., 1999, Biochemical Journal 338, 515-521). As compared with starch which is isolated from corresponding wild-type plants, starch from these plants exhibits a relative shift of the amylopectin side chains from relatively long chains to short chains (Lloyd et al., 1999, Biochemical Journal 338, 515-521), an increased content of phosphate, no change in the amylose content (Abel et al., 1996, The Plant Journal 10(6), 9891-9991) and a reduced final viscosity in the RVA analysis (Abel, 1995, Berlin Free University dissertation).

[0010] Plants in which the activity of branching enzyme I (BEI) is reduced have also been described (Kossmann et al., 1991, Mol. Gen. Genet. 230, 39-44; Safford et al., 1998, Carbohydrate Polymers 35, 155-168; WO 92/14827). Safford et al. (1998, see above) report that corresponding potatoes produce a starch which has a slightly altered amylose/amylopectin ratio. Nor does the degree of branching of the amylopectin differ significantly from that of a starch which is isolated from wild-type potatoes. However, the starch-bound phosphate content is slightly increased.

[0011] WO 01/19975 describes plants in which the GBSSI and the SSII and/or SSIII activities are reduced. As compared with starch from wild-type potatoes, starch from potatoes having reduced activities of GBSSI, SSII and SSIII exhibit a lower amylose content, altered swellability and pasting properties and higher freeze/thaw stability.

[0012] WO 01/12782 describes plants in which both the GBSSI activity and the BEI activity are reduced. Starch from these potato plants exhibits a reduced amylose content as compared with potato starch from wild-type plants and an elevated phosphate content and/or a reduced pasting temperature in the RVA analysis as compared with potato starch from plants having the waxy phenotype.

[0013] WO 00/08184 describes, inter alia, plants in which both the SSIII activity and the BEI activity are reduced. Starch from these plants exhibits a markedly elevated phosphate content as compared with starch from wild-type plants.

[0014] The present invention is based on the object of making available potato amylopectin starches having novel properties, novel plant cells and/or plants which produce the starches, as well as means and methods for generating said plant cells and/or plants.

[0015] This object is achieved by the provision of the embodiments which are described in the patent claims.

[0016] The present invention relates to potato starches which have an amylose content, as measured by the method of Hovenkamp-Hermelink et al. (1988, Potato Research 31, 241-246), of less than 10% by weight, a phosphate content in the C6 position, as measured by method b) "Determining the phosphate content in the C6 position" described in section "General methods", 2. Starch analysis, of between 35 and 100 nmol of phosphate per milligram of starch (dry weight), and a content of side chains, as measured by method f) "Using gel permeation chromatography to analyze the side chain distribution in total starch" described in section "General methods", 2. Starch analysis, having a DP of from 12 to 19 which is increased to 125%-200%, as compared with that in potato starch from corresponding wild-type potato plants.

[0017] The present invention furthermore discloses potato starches which have an amylose content, as measured by the method ("General methods") of Hovenkamp-Hermelink et al. (1988, Potato Research 31, 241-246), of less than 10% by weight, a phosphate content in the C6 position of between 35 and 100 nmol of phosphate per mg of starch (dry weight) and a total phosphate content to phosphate content in the C6 position ratio of 1.10-1.60.

[0018] The present invention furthermore discloses potato starches which have an amylose content, as measured by the method ("General methods") of Hovenkamp-Hermelink et al. (1988, Potato Research 31, 241-246), of less than 10% by weight, a phosphate content in the C6 position of between 35 and 100 nmol of phosphate per mg of starch (dry weight) and a shear stability of 58% to 80%, in particular of 60% to 78%, preferably of 66% to 77%, particularly preferably of 67% to 75%.

[0019] The present invention furthermore discloses potato starches which have an amylose content, as measured by the method ("General methods") of Hovenkamp-Hermelink et al. (1988, Potato Research 31, 241-246), of less than 10% by weight and a peak viscosity determined by the Rotovisko method of 332 SKT to 500 SKT, in particular of 345 SKT - 450 SKT, preferably of 360 SKT to 420 SKT and particularly preferably of 370 SKT to 400 SKT.

[0020] The present invention furthermore discloses potato starches which have an amylose content, as measured by the method ("General methods") of Hovenkamp-Hermelink et al. (1988, Potato Research 31, 241-246), of less than 10% by weight, a phosphate content in the C6 position of between 35 and 100 nmol of phosphate per mg of starch (dry weight) and a peak viscosity determined by the Rotovisko method of 332 SKT to 500 SKT, in particular of 345 SKT - 450 SKT, preferably of 360 SKT to 420 SKT and particularly preferably of 370 SKT to 400 SKT.

[0021] In connection with the present invention, the amylose content is determined using the method of Hovenkamp-Hermelink et al. (1988, Potato Research 31, 241-246), which is described below for potato starch. This method can also be applied to starches which are isolated from other plant species. Methods for isolating starches are known to the skilled person and are described in detail below in the "General methods" section.

[0022] In connection with the present invention, the term "phosphate content in the C6 position" is to be understood as meaning the content of phosphate groups which are covalently bonded to carbon atom position 6 in the glucose monomers in the starch. In principle, the C2, C3 and C6 positions in the glucose units can be phosphorylated in the

starch in vivo. In connection with the present invention, the phosphate content in the C6 position (= C6-P content) is determined by way of a glucose-6-phosphate determination using the opticoenzymic test which is described below ("General methods: determining the phosphate content in the C6 position").

[0023] In connection with the present invention, the term "total phosphate content" is to be understood as meaning the quantity of starch phosphate which is in total covalently bonded to glucose molecules in the starch. In connection with the present invention, the total phosphate content is determined using the method which is described below ("General methods: determining the total phosphate content").

[0024] Starch of the present invention, shows an "elevated content of side chains having a DP of from 12 to 19" which means an increase in the proportion of side chains in the starch having a DP (= degree of polymerization) of from 12 to 19 to 125%-200%, preferably to 130%-180%, and particularly preferably to 140%-160%, as compared with the proportion of side chains having a DP of from 12 to 19 in potato starch which is isolated from corresponding wild-type potato plants (100%).

[0025] In connection with the present invention, the side chain distribution in the starch is determined as described below in the section entitled "General methods: using gel permeation chromatography to analyze the side chain distribution in total starch".

[0026] In connection with the present invention, the term "wild-type potato plant cell" means that the cells are potato plant cells which were used as the starting material for producing the plant cells according to the invention, i.e. their genetic information corresponds, apart from the genetic modification which has been introduced, to that of a plant cell according to the invention. In connection with the present invention, the term "wild-type potato plant" means that the plants are plants which were used as the starting material for producing the plants according to the invention which are described below, i.e. their genetic information corresponds, apart from the genetic modification which has been introduced, to that of a plant according to the invention.

[0027] In connection with the present invention, the term "corresponding" means that, when comparing several objects, the objects in question, which are being compared with each other, were maintained under identical conditions. In connection with the present invention, the term "corresponding" means, with regard to wild-type plant cells or wild-type plants, in particular, that the plant cells or plants which are being compared with each other were grown under identical culture conditions and that they are of the same (culture) age.

[0028] In a preferred embodiment of the invention, the potato starches according to the invention have an amylose content, as measured using the method of Hovenkamp-Hermelink et al. (1988, Potato Research 31, 241-246), of less than 5% by weight, particularly preferably of less than 3% by weight.

[0029] In another preferred embodiment of the invention, the potato starch according to the invention has an elevated phosphate content in the C6 position of 40-85 nmol of C6-P per mg of starch, particularly preferably of 45-70 nmol of C6-P per mg of starch, very particularly preferably of 50-65 nmol of C6-P per mg of starch.

[0030] In another preferred embodiment of the invention, the potato starch according to the invention exhibits an elevated phosphate content as compared with that in potato starch derived from corresponding wild-type potato plants. In connection with the present invention, the term "elevated phosphate content" means that the phosphate content in the C6 position in the starch according to the invention is elevated, in particular elevated by 415%-520%, preferably by 430%-500%, and particularly preferably by 440%-490%, as compared with that in starch which is derived from corresponding wild-type plant cells or plants.

[0031] In another embodiment of the invention, the potato starch according to the invention exhibits an "elevated content of side chains having a DP of <12". In connection with the present invention, this means an increase in the proportion of the sum of side chains in the starch having a DP (degree of polymerization) of less than 12 to 130%-170%, preferably to 140%-160%, and particularly preferably to 145%-155%, as compared with the proportion of side chains having a DP of less than 12 (100%) in potato starch which is derived from corresponding wild-type potato plants.

[0032] In another embodiment of the invention, the potato starch according to the invention exhibits an "elevated content of side chains having a DP of 20-25". In connection with the present invention, this means an increase in the proportion of the sum of side chains in the starch having a DP (= degree of polymerization) of 20-25 to 132%-160%, preferably to 136%-150%, and particularly preferably to 139%-148%, as compared with the proportion of side chains having a DP of 20-25 (100%) in potato starch which is derived from corresponding wild-type potato plants.

[0033] In another embodiment of the invention, the potato starch according to the invention exhibits a "reduced content of side chains having a DP of 63-123". In connection with the present invention, this means a reduction in the proportion of the sum of side chains in the starch having a DP (= degree of polymerization) of 63-123 to 50%-95%, preferably to 65%-90%, and particularly preferably to 73%-85%, as compared with the proportion of side chains having a DP of 63-123 (100%) in potato starch which is derived from corresponding wild-type potato plants.

[0034] In another embodiment of the invention, the potato starches according to the invention exhibit a "reduced content of side chains having a DP of >123". In connection with the present invention, this means a reduction in the proportion of the sum of side chains in the starch having a DP (= degree of polymerization) of greater than 123 to 0.1%-3.8%, preferably to 0.3%-3.0%, and particularly preferably to 0.5%-2.5%, as compared with the proportion of side chains

having a DP greater than 123 (100%) in potato starch which is derived from corresponding wild-type potato plants.

[0035] In another preferred embodiment of the invention, the potato starches according to the invention exhibit a total phosphate content to phosphate content in the C6 position ratio of 1.20-1.50, particularly preferably of 1.30-1.40.

[0036] In another embodiment of the invention, the potato starches according to the invention exhibit high freeze/thaw stability.

[0037] In connection with the present invention, the term "high freeze/thaw stability" means a freeze/thaw stability of at least 60%, in particular of at least 70%, preferably of at least 80%, and particularly preferably of at least 95%. In connection with the present invention, the freeze/thaw stability is determined using the method which is described below ("General methods").

[0038] In another embodiment of the invention, the potato starches according to the invention exhibit a high degree of heat stability.

[0039] In connection with the present invention, the term "high degree of heat stability" means a heat stability of at least 30%, in particular of at least 40%, and preferably of at least 50%. In connection with the present invention, the heat stability is determined using the method which is described below ("General methods").

[0040] In another embodiment of the invention, the potato starches according to the invention exhibit a high degree of shear stability.

[0041] In connection with the present invention, the term "high degree of shear stability" means a shear stability of 58% to 80%, in particular of 60% to 78%, preferably of 66% to 77%, particularly preferably of 67% to 75%. In connection with the present invention, the shear stability is determined using the method which is described below ("General methods: Method k).

[0042] In another embodiment of the invention, the potato starches according to the invention exhibit a viscosity behavior (e.g. pasting temperature, final viscosity) which is altered as compared with that of potato starch which is derived from corresponding wild-type potato plants. In connection with the present invention, the viscosity properties are determined using the RVA or the Rotovisko method which is described below ("General methods").

[0043] In another embodiment of the invention, the potato starches according to the invention exhibit an increased peak viscosity determined by the Rotovisko method ("General methods: Method I").

[0044] In connection with the present invention, the term "increased peak viscosity determined by the Rotovisko method" means an increase of the peak viscosity by 23% to 70%, in particular by 27% to 60%, preferably by 35% to 55%, particularly preferably by 40% to 50% compared with the peak viscosity of potato starch which is derived from corresponding wild-type potato plants (100%).

[0045] In another embodiment of the invention, the potato starches according to the invention exhibit a peak viscosity determined by the Rotovisko method of 332 SKT to 500 SKT, in particular of 345 SKT - 450 SKT, preferably of 360 SKT to 420 SKT and particularly preferably of 370 SKT to 400 SKT.

[0046] In another embodiment of the invention, the potato starches according to the invention exhibit a DSC peak temperature which is altered as compared with that of potato starch which is derived from corresponding wild-type potato plants. In connection with the present invention, the DSC peak temperature is determined using the method which is described below ("General methods").

[0047] In another embodiment of the invention, the potato starches according to the invention exhibit a gel strength which is reduced as compared with that of potato starch which is derived from corresponding wild-type potato plants. In connection with the present invention, the gel strength is determined using the method which is described below ("General methods").

[0048] In another embodiment of the invention, the potato starches according to the invention exhibit a gel strength of 1,0 g to 10,0 g , in particular of 3,5 g to 7,5 g, preferably of 3,7 g to 6,5 g and particularly preferably of 4,0 g to 6,0 g.

[0049] The potato starches according to the invention are preferably native potato starches. In connection with the present invention, the term "native starch" means that methods known to the skilled person are used to extract the starch from plants or starch-storing parts of plants without the extracted starch being chemically modified following the extraction.

[0050] Furthermore, the present invention discloses to a method for the manufacture of the (potato) starch according to the invention, including the step of extracting the starch from a plant cell or from a plant, from propagation material of such a plant and/or from harvestable plant parts of such a plant, preferably from starch-storing parts of such a plant. Preferably, such a method also includes the step of harvesting the cultivated plants or plant parts and/or the propagation material of these plants before the extraction of the starch and, further, particularly preferably the step of cultivating plants before harvesting.

[0051] The skilled person is familiar with methods for isolating starch from plants or from starch-storing parts of plants. Methods for extracting the starch from different starch-storing plants have also been described, e.g. in Starch: Chemistry and Technology (eds.: Whistler, BeMiller and Paschall (1994), 2nd edition, Academic Press Inc. London Ltd., ISBN 0-12-746270-8; see, e.g. Chapter XII, pages 412-468; corn and sorghum starches: preparation; by Watson; Chapter XIII, pages 469-479: tapioca, arrowroot and sago starches: preparation; by Corbishley and Miller; Chapter XIV, pages 479-490: potato starch: preparation and uses; by Mitch; Chapter XV, pages 491 to 506: wheat starch: preparation,

modification and uses; by Knight and Oson; and Chapter XVI, pages 507 to 528: rice starch: preparation and uses; by Rohmer and Klem; corn starch: Eckhoff et al., Cereal Chem. 73 (1996), 54-57, as a rule corn starch is extracted on an industrial scale using what is termed "wet milling".

[0052] The present invention also discloses potato starches according to the invention which possess one or more of the above-described properties. That is, this invention description discloses any combination of the following starch properties: amylose content or amylose/amylopectin ratio, phosphate content, side chain distribution, freeze/thaw stability and heat stability. Any combinations of two, three, four, five, six, seven, eight, nine and all the properties are to be regarded as being disclosed.

[0053] The starches according to the invention exhibit properties which appear to make them particularly suitable for being used in processes in which a pronounced ability to swell, a high degree of freeze/thaw stability and/or a high charge density are advantageous. These requirements apply, for example, to thickeners in the foodstuffs industry, especially when these thickeners are frozen for storage or processing and/or a particularly high thickening efficiency is desirable.

[0054] Because of their unusually high charge density, which is to be attributed to the covalently bonded phosphate groups, and their low-viscosity thickening, the starches according to the invention are particularly well suited for being used in the paper industry. The high charge density is advantageous since it makes it possible to produce frequently employed amphoteric starches in what is only a one-step derivatization reaction and to a large extent dispense with additional derivatization reactions for introducing negative charges into the starches.

[0055] Standard methods, which are known to the skilled person, can be used to chemically and/or physically modify the potato starches according to the invention, preferably native potato starches, after they have been extracted from the potato tubers.

[0056] The skilled person knows that the properties of native potato starch can be altered by, e.g., physical (e.g. thermal or mechanical) and/or chemical derivatization and/or breakdown products of the starch (e.g. dextrins) which are obtained by enzymic, acid-hydrolytic or thermal degradation. The starches which are obtained in this connection, and which are to be designated "derivatized potato starches" in connection with the present invention, are particularly suitable for a variety of applications. The native potato starches according to the invention are better suited than are conventional potato starches (derived from wild-type potato plants) for being used as starting substance for preparing the derivatized potato starches because this starting substance exhibits, for example, a higher proportion of reactive functional groups, as a result of the higher content of covalently bonded starch phosphate, is more strongly hydrophilic and is more accessible to chemical agents.

[0057] The present invention therefore also relates to derivatized potato starches which contain the, preferably native, potato starches according to the invention and to methods for preparing such a derivatized starch, in which methods potato starch according to the invention, which is preferably native, is subsequently, i.e. after having been extracted from the potato tuber, chemically and/or physically modified, preferably in vitro.

[0058] The derivatized starch according to the invention is, in particular, heat-treated starch. The present invention preferably relates to acid-modified starch which has preferably been treated with acid, preferably with hydrochloric acid (at a concentration of up to 1 M) in an aqueous system at temperatures of up to 50°C.

[0059] In another embodiment, the present invention relates to derivatized starches which were obtained by subjecting the, preferably native, potato starch according to the invention to a temperature treatment in a dry system, preferably at temperatures of from preferably 120°C to 140°C.

[0060] In another embodiment, the derivatized starches are starch ethers, in particular starch alkyl ethers, O-allyl ethers, hydroxyalkyl ethers, O-carboxymethyl ethers, nitrogen-containing starch ethers, phosphate-containing starch ethers or sulfur-containing starch ethers.

[0061] In another embodiment, the derivatized starches are crosslinked starches.

[0062] In another embodiment, the derivatized starches are starch graft polymers.

[0063] In another embodiment, the derivatized starches are oxidized starches.

[0064] In another embodiment, the derivatized starches are starch esters, in particular starch esters which were introduced into the starch using organic acids. The starch esters are particularly preferably phosphate, nitrate, sulfate, xanthate, acetate or citrate starches.

[0065] The derivatized starches according to the invention are suitable for a variety of uses in the pharmaceutical industry and in the foodstuffs and/or nonfoodstuffs spheres. Methods for preparing derivatized starches according to the invention are known to the skilled person and are adequately described in the general literature. A review regarding the preparation of derivatized starches can be found, for example, in Orthoefer (in: Corn, Chemistry and Technology, 1987, eds. Watson and Ramstad, Chapter 16, 479-499).

[0066] The present invention likewise relates to derivatized starch which can be obtained using the method according to the invention for preparing a derivatized starch.

[0067] The present disclosure furthermore relates to the use of potato starches according to the invention, which are preferably native, for preparing derivatized potato starch.

[0068] The potato starches according to the invention are suitable, in native or derivatized form, for a variety of uses in the foodstuffs or nonfoodstuffs sphere.

[0069] In principle, the possibilities for using the starch can be divided into two large areas. One of the areas comprises the hydrolysis products of the starch, principally glucose and glucan building blocks, which are obtained using enzymic or chemical methods. They serve as starting compounds for further chemical modifications and processes such as fermentation. In this connection, the simplicity and economical implementation of a hydrolysis process can be of importance for reducing the cost. At present, the process proceeds essentially enzymatically using amyloglucosidase. It will be possible to conceive of saving costs by making less use of enzymes. A change in the structure of the starch, e.g. an increase in the surface of the granule, easier digestibility as a result of a lower degree of branching, or a steric structure which limits accessibility for the enzymes employed, could bring this about.

[0070] The other area, in which, because of its polymeric structure, the starch is used as what is termed native starch, divides into two further areas of use:

1. Foodstuffs industry

Starch is a classical additive for many foodstuffs in which it essentially takes on the function of binding aqueous additives or brings about an increase in viscosity or else an increase in gel formation. Important characteristic features are the flowing and sorption behaviors, the swelling and pasting temperatures, the viscosity and the thickening efficiency, the solubility of the starch, the transparency and paste structure, the heating, shearing and acid stabilities, the tendency to retrogradation, the film-forming ability, the freeze/thaw stability, the digestibility and the ability to form complexes with, for example, inorganic or organic ions.

2. Non-foodstuffs industry

In this large area, the starch can be used as an auxiliary for different production processes or as an additive in industrial products. The paper and paperboard industry is to be mentioned, in particular, in connection with using the starch as an auxiliary. In this connection, the starch is first and foremost for retardation (retention of solids), for binding filler and fines particles, as a stabilizer and for dewatering. In addition to this, the favorable properties of the starch are exploited in relation to stiffness, hardness, rattle, feel, shine, glaze, plybond strength and the surfaces.

2.1 Paper and paperboard industry

Four areas of application, namely surface, coating, pulp and spraying are to be distinguished within the paper manufacturing process.

The demands placed on the starch with regard to surface treatment are essentially a high degree of brightness, an appropriate viscosity, high viscosity stability, good film formation and low dust formation. When being used in coating, the solids content, an appropriate viscosity, high binding ability and high pigment affinity are of importance. When being used as a pulp additive, rapid, uniform and loss-free dispersion, high mechanical stability and complete retention in the paper web are of importance. When the starch is being used in the spraying area, an appropriate solids content, a high viscosity and a high binding ability are likewise of importance.

2.2 Adhesives industry

A large area for using the starches is constituted by the adhesives industry, where the possibilities of employment are divided into four constituent areas: use as pure starch glue, use in connection with starch glues which are prepared using special chemicals, use of starch as a substance added to synthetic resins and polymer dispersions, and use of starches as extenders for synthetic adhesives. 90% of the starch-based adhesives are used in the areas constituting corrugated board production, production of paper sacks and bags, production of materials for bonding paper and aluminum, production of cardboard articles, and production of remoistening glue for envelopes, postage stamps, etc.

2.3 Textile industry and textile care product industry

The area of textile production and textile care product production represents a large field for using the starches as auxiliaries and additives. The following four areas of use can be distinguished within the textile industry: the use of starch as a sizing material, i.e. as an auxiliary for smoothing and strengthening burring behavior, for protecting against the tractive forces which act during weaving, and for increasing the abrasion resistance in connection with weaving; starch as an agent for textile finishing, especially after quality-impairing pretreatments such as bleaching, dyeing, etc.; starch as a thickener in connection with producing pigment pastes for preventing dye diffusions; and starch as a substance added to warping agents for sewing cottons.

2.4 Building material industry

The fourth area of employment is the use of the starches as additives for building materials. An example is the

production of gypsum plasterboards in which the starch which is mixed in the gypsum slurry forms a paste with the water, diffuses to the surface of the gypsum panel, where it binds the paperboard to the panel. Other areas of employment are admixing to rendering fibers and mineral fibers. In the case of ready-mixed concrete, starch products are used for delaying setting.

2.5 Soil stabilization

Another market for the starch presents itself in the case of the production of soil stabilization agents, which are employed for temporarily protecting the soil particles from water in association with earth moving operations. While, according to present day knowledge, the erosion-reducing and incrustation-reducing effects of combination products composed of starch and polymer emulsions are on a level with those of previously employed products, their price is markedly lower than that of these products.

2.6 Use in plant protection products and fertilizers

One area of employment lies in using the starch in plant protection products for the purpose of modifying the specific properties of the preparations. Thus, the starch can be used for improving the wetting properties of plant protection products and fertilizers, for the metered release of the active compounds, for converting liquid, volatile and/or malodorous active compounds into microcrystalline, stable and formable substances, for mixing incompatible compounds and for extending the duration of action by reducing decomposition.

2.7 The pharmaceuticals, medicines and cosmetics industry

The area of the pharmaceuticals, medicines and cosmetics industry constitutes another area of employment. In the pharmaceutical industry, the starch can be used as a binder for tablets or for binder dilution in capsules. The starch can also be used as a tablet disintegrant since it absorbs liquid after swallowing and after a short time swells to such an extent that the active compound is released. For reasons of quality, medicinal glidants and wound powders are based on starch. In the sphere of cosmetics, starches are, for example, employed as carriers of powder additives, such as perfumes and salicylic acid. Toothpastes constitute a relatively area for using the starch.

2.8 Addition of starch to coal and briquettes

One area of employment is that of using the starch as a substance which is added to coal and briquettes. A starch additive can be used to agglomerate or briquette coal in a quantitatively high-grade manner, thereby preventing any premature decomposition of the briquettes. The addition of starch is between 4 and 6% in the case of grilling coal and between 0.1 and 0.5% in the case of calorized coal. Starches are also gaining in importance as binders since their addition to coal and briquettes can markedly reduce the release of harmful substances.

2.9 Ore and coal slurry dressing

The starch can also be used as a flocculant in connection with ore and coal slurry dressing.

2.10 Foundry auxiliary

Another area of use is as a substance which is added to foundry auxiliaries. In a variety of casting processes, there is a need for cores which are produced from sands to which binder has been added. The binder which is nowadays mainly used is bentonite to which modified starches, usually swelling starches, have been added.

The purpose of adding the starch is to increase the flow resistance and to improve binding strength. In addition to this, the swelling starches can exhibit other requirements in relation to production technology, such as dispersible in cold water, rehydratable, readily miscible in sand and high water-binding capacity.

2.11 Use in the rubber industry

In the rubber industry, the starch can be used for improving technical and optical quality. The reasons in this connection are the improvement in the surface shine, the improvement in the feel and the appearance (for this, starch is dusted onto the tacky gummed areas of rubbers prior to cold vulcanization) and the improvement in the printability of the rubber.

2.12 Use as a drilling auxiliary

Another possible application for the starches according to the invention is in the area of extracting raw materials using drills. Thus, in connection with extracting crude oil, for example, it is necessary to use auxiliaries and/or lubricants which prevent the drill or the drilling gear from overheating.

2.13 Production of leather substitutes

Another opportunity for marketing the modified starches is in connection with the production of leather substitutes.

2.14 Starch in synthetic polymers

The following areas of application are apparent in the plastics sector: the integration of secondary starch products into the finishing process (starch is only a filler, there is no direct bond between the synthetic polymer and the starch) or alternatively, the integration of secondary starch products into the production of polymers (the starch and the polymer enter into a stable bond).

[0071] When compared with other substances such as talc, it is uncompetitive to use the starch simply as a filler. The situation is different when the specific properties of the starch come into play and the property profiles of the final products are markedly altered as a result. An example of this is the use of starch products in the finishing of thermoplastics such as polyethylene. In this connection, the starch and the synthetic polymer are combined, by being coexpressed in a ratio of 1:1, into a "masterbatch", from which various products are produced using granular polyethylene and conventional process technology. The integration of starch into polyethylene films makes it possible to achieve an increase in substance permeability in the case of hollow bodies, an improvement in water vapor permeability, an improvement in antistatic behavior, an improvement in antiblocking behavior and an improvement in printability when using aqueous pigments.

[0072] Another possibility is that of using the starch in polyurethane foams. By means of adapting the starch derivatives and optimization with regard to process technology, it is possible to control the reaction between synthetic polymers and the hydroxyl groups of the starches in a selective manner. This results in polyurethane films which are given the following property profiles as a result of starch being used: a reduction in the thermal expansion coefficient, a reduction in the shrinkage behavior, an improvement in the pressure/tension behavior, an increase in water vapor permeability without any change in water uptake, a reduction in flammability and cracking density, no dripping-off of combustible parts, freedom from halogens and diminished aging. Disadvantages which still exist at present are a reduction in compression strength and a reduction in impact strength.

[0073] Product development is by now no longer restricted to films. Solid plastic products, such as pots, plates and bowls, having a starch content of more than 50% can also be produced. Furthermore, starch/polymer mixtures are to be judged as being advantageous since they exhibit a very much higher degree of biodegradability.

[0074] Furthermore, because of their extreme ability to bind water, starch graft polymers have become exceptionally important. These polymers are products having a starch backbone and a side lattice of a synthetic monomer which is grafted on in accordance with the principle of the free-radical chain mechanism. The starch graft polymers which are nowadays available are characterized by an improved ability to bind and retain up to 1000 g of water per g of starch in association with high viscosity. The areas in which these superabsorbers can be used have expanded greatly in recent years and lie in the hygiene sphere, involving products such as diapers and paddings, and in the agricultural sector, for example in connection with seed pelleting.

[0075] Factors which are crucially relevant for using the novel starches are, on the one hand, the structure, water content, protein content, lipid content, fiber content, ash/phosphate content, amylose/amylopectin ratio, molar mass distribution, degree of branching, granule size and shape, and crystallinity, and also, on the other hand, the properties which lead to the following features: flow and sorption behavior, pasting temperature, viscosity, thickening efficiency, solubility, paste structure and transparency, heat, shearing and acid stability, retrogradation tendency, gel formation, freeze/thaw stability, complex formation, iodine binding, film formation, adhesive strength, enzyme stability, digestibility and reactivity.

[0076] The potato starch according to the invention, which is preferably native, can be prepared by isolating it from genetically modified potato plants in which the genetic modification leads to a reduction in the GBSSI, SSIII and BEI activities and to a reduction in the expression of the gene specified under SEQ ID NO 11 or SEQ ID NO 13, as compared with the activities of the corresponding wild-type potato plant cells or wild-type potato plants.

[0077] The present disclosure therefore also relates to plant cells and plants which are genetically modified, with the genetic modification leading to a reduction in the GBSSI, SSIII and BEI activities, and to a reduction in the expression of the gene specified under SEQ ID NO 11 or SEQ ID NO 13, as compared with the activities of corresponding wild-type plant cells or wild-type plants.

[0078] In connection with the present invention, the term "GBSSI" is to be understood as meaning any enzyme which belongs to the isoform I class of starch granule-bound starch synthases (EC 2.4.1.21).

[0079] In connection with the present invention, the term "GBSSI gene" is to be understood as meaning a nucleic acid molecule or polynucleotide (cDNA or DNA) which encodes GBSSI. Polynucleotides encoding GBSSI have been described in the case of a variety of plant species, such as corn (Genbank Acc. Nos. AF079260, AF079261), wheat (Genbank Acc. Nos. AB019622, AB019623, AB019624), rice (Genbank Acc. Nos. AF092443, AF09244, AF031162), barley (Genbank Acc. Nos. X07931, X07932), and potato (Genbank Acc. No. X58453). In one embodiment of the invention, the GBSSI gene exhibits an identity of at least 70%, in particular of at least 80%, of at least 90%, preferably of at least 95%, with the coding region of the nucleotide sequence which is depicted in SEQ ID NO 6. In a particularly preferred embodiment, the GBSSI gene is, in connection with the present invention, a nucleic acid molecule (cDNA or DNA) which encodes potato plant GBSSI; particular preference is given to the GBSSI gene which is specified under SEQ ID NO 6.

[0080] In connection with the present invention, the term "SSIII" is to be understood as meaning a particular class of soluble starch synthases (ADP-glucose 1,4-α-D-glucan 4-α-D-glucosyltransferase; EC 2.4.1.21). Soluble starch synthases catalyze a glycosylation reaction in which glucose residues of the substrate ADP-glucose are transferred to α-1,4-linked glucan chains with the formation of an α-1,4 linkage (ADP-glucose + {(1,4)-α-D-glucosyl}(N) <=> ADP + {(1,4)-α-D-glucosyl}(N+1)).

[0081] SSIIIs are described, for example, in Marshall et al. (1996, The Plant Cell 8, 1121-1135), Li et al. (2000, Plant Physiology 123, 613-624), Abel et al. (1996, The Plant Journal 10(6), 981-991) and in WO 00/66745. The structure of SSIIIs frequently exhibits a sequence of particular domains and possesses a signal peptide, for transport into plastids, at its N terminus. There then follow, in the direction of the C terminus, an N-terminal region, an SSIII-specific region and a catalytic domain (Li et al., 2000, Plant Physiology 123, 613-624). Further analyses based on primary sequence comparisons (http://hits.isb-sib.ch/cgi-bin/PFSCAN) have shown that potato-derived SSIII protein exhibits what is termed a carbohydrate binding domain (CBM). This domain (Pfam motif cbm 25 = SEQ ID NO 3) comprises amino acids 377 to 437 of the potato SSIII protein sequence depicted in SEQ ID NO 2. In connection with the present invention, an SSIII protein is to be understood as being a protein which exhibits an identity of at least 70%, preferably of at least 80%, particularly preferably of at least 90%, very particularly preferably of at least 95%, with the sequence depicted in SEQ ID NO 2.

[0082] In connection with the present invention, the term "SSIII gene" is to be understood as meaning a nucleic acid molecule (DNA or cDNA) which encodes an SSIII protein. Nucleic acid molecules encoding SSIIIs have been described in the case of a variety of plant species, for example the potato (Abel et al., 1996, The Plant Journal 10(6), 981-991). In connection with the present invention, an "SSIII gene" is to be understood as meaning a nucleic acid molecule which exhibits an identity of least 70%, in particular of at least 80%, preferably of at least 90%, very particularly preferably of at least 95%, with the coding region of the sequence depicted in SEQ ID NO 1. In a particularly preferred embodiment, the SSIII gene is, in connection with the present invention, a nucleic acid molecule (cDNA or DNA) which encodes potato plant SSIII; particular preference is given to the potato SSIII gene which is specified under SEQ ID NO 1.

[0083] In connection with the present invention, the term "BEI" is to be understood as meaning an isoform I branching enzyme (BE) (α-1,4-glucan: α-1,4-glucan 6-glycosyltransferase; E.C. 2.4.1.18), which catalyzes a transglycosylation reaction in which α-1,4-linkages in an α-1,4-glucan donor are hydrolyzed and the α-1,4-glucan chains which are released in this connection are transferred to an a-1,4-glucan acceptor chain and, in association with this, converted into α-1,6 linkages. BEI is preferably derived from potato plants.

[0084] In this connection, the designation of the isoforms follows the nomenclature proposed by Smith-White and Preiss (Smith-White and Preiss, 1994, Plant Mol. Biol. Rep. 12, 67-71; Larsson et al., 1998, Plant Mol. Biol. 37, 505-511). This nomenclature is based on all enzymes which exhibit higher homology (identity) at the amino acid level with corn BEI (GenBank Acc. No. D11081; Baba et al., 1991, Blochem. Biophys. Res. Commun. 191 (1), 87-94; Kim et al., 1998, Gene 216, 233-243) than with corn BEII (Genbank Acc. Nos. AF072725, U65948) being designated isoform I branching enzymes or BEIs for short.

[0085] In connection with the present invention, the term "BEI gene" is to be understood as meaning a nucleic acid molecule or polynucleotide (cDNA or DNA) which encodes BEI. Polynucleotides encoding BEIs have been described in the case of a variety of plant species, for example in the case of corn (Genbank Acc. Nos. D11081, AF072724), rice (Genbank Acc. No. D11082) and potato. Various forms of the potato BEI gene or potato BEI have, for example, been described by Khoshnoodi et al. (1996, Eur. J. Biochem. 242 (1), 148-155, Genbank Acc. No. Y08786) and by Kossmann et al. (1991, Mol. Gen. Genet. 230, 39-44).

[0086] In one embodiment of the invention, the BEI gene exhibits an identity of at least 70%, in particular of at least 80%, of at least 90%, preferably at least 95%, with the coding region of the nucleotide sequence depicted in SEQ ID NO 4. In a particularly preferred embodiment, the BEI gene is, in connection with the present invention, a nucleic acid molecule (cDNA or DNA) which encodes potato plant BEI; particular preference is given to the BEI gene specified under SEQ ID NO 4. In potato plants, the BEI gene is principally expressed in the tubers and hardly at all in the leaves (Larsson et al., 1998, Plant Mol. Biol. 37, 505-511).

[0087] In the context of the definitions of the terms "GBSSI gene", "SSIII gene" and/or "BEI gene", the "Genbank Acc" numbers and literature reference citations refer to specific polynucleotide sequences which encode the corresponding enzymes. Embodiments of the present invention in which polynucleotides having the sequences specified in the citations can be used are described below. In this connection, the invention is naturally not restricted to using such precisely described sequences or parts of these sequences. It is also possible, for example, to use polynucleotides which exhibit an identity of at least 80%, preferably at least 90%, particularly preferably of at least 95%, and very particularly preferably of at least 98%, with the sequences which are specified.

[0088] Genes which exhibit the nucleotide sequence specified under SEQ ID NO 11 or SEQ ID NO 13, and their corresponding proteins which exhibit the amino acid sequence specified under SEQ ID NO 12 or SEQ ID NO 14, are involved in starch biosynthesis in plants. The amino acid sequences of these proteins exhibit homology with *Arabidopsis thaliana* proteins (GenBank Acc. No: BAB02827), which are described there as being branching enzyme-like proteins.

In connection with the present invention, it has been found, surprisingly, that potato plants which exhibit reduced expression of the gene specified under SEQ ID NO 11 or SEQ ID NO 13, and exhibit a reduction in GBSSI, SSIII and BEI activities, produce the, preferably native, potato starches according to the invention. It can be concluded from this that the gene specified under SEQ ID NO 11 or SEQ ID NO 13, or the protein which is encoded by this gene and has the amino acid sequence specified under SEQ ID NO 12 or SEQ ID NO 14, is involved, in potato plants, in the synthesis of the amylopectin side chains or in the phosphorylation of starch.

[0089] In connection with the present invention, the term "identity" is to be understood as meaning the number of amino acids/nucleotides which concur with those of other proteins/nucleic acids, expressed as a percentage. The identity is preferably determined using computer programs. If sequences which are being compared with each other are of differing lengths, the identity is to be determined such that the number of amino acids which the shorter sequence has in common with the longer sequence determines the percentage identity. The identity is preferably determined using the ClustalW computer program (Thompson et al., 1994, Nucleic Acids Research 22, 4673-4680), which is known and available to the public. ClustalW is made publicly available by Julie Thompson (Thompson@EMBL-Heidelberg.de) and Toby Gibson (Gibson@EMBL-Heidelberg.de), European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany. ClustalW can also be downloaded from a variety of internet sites, including from IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire, B.P.163, 67404 Illkirch Cedex, France; (ftp://ftp-igbmc.u.-strasbg.fr/pub/) and from EBI (ftp://ftp.ebi.ac.uk/pub/software/) and from all mirrored internet sites belonging to the EBI (European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 ISD, UK).

[0090] Preference is given to using version 1.8 of the ClustalW computer program for determining the identity between the proteins which are described here and other proteins. The following parameter settings are to be used in this connection: KTUPLE=1, TOPDIAG=5, WINDOW=5, PAIRGAP=3, GAPOPEN=10, GAPEXTEND=0.05, GAPDIST=8, MAXDIV=40, MATRIX=GONNET, ENDGAPS(OFF), NOPGAP, NOHGAP.

[0091] Preference is given to using version 1.8 of the ClustalW computer program for determining the identity between the nucleotide sequences of the nucleic acid molecules which are described herein and the nucleotide sequences of other nucleic acid molecules. The following parameter settings are to be used in this connection: KTUPLE=2, TOPDIAGS=4, PAIRGAP=5, DNAMATRIX:IUB, GAPOPEN=10, GAPEXT=5, MAXDIV=40, TRANSITIONS: unweighed.

[0092] One option for finding similar sequences is to carry out sequence database searches. In the searches, one or more sequences are predetermined to be what is termed the query. Statistical computer programs are then used to compare this query sequence with sequences which are contained in the chosen databases. Such database searches (blast searches) are known to the skilled person and can be carried out using the databases provided by different suppliers. If such a database search is carried out using the NCBI (National Center for Biotechnology Information, http://www.ncbi.nim.nih.gov/) database, the standard settings which are predetermined for the given comparison query should then be used. In the case of protein sequence comparisons (blastp), these settings are as follows: Limit entrez = not activated; Filter = low complexity activated; Expect value = 10; word size = 3; Matrix = BLOSUM62; Gap costs: Existence = 11, Extension = 1. Such a search also results in the percentage identity between the query sequence and the similar sequences which are found in the databases being presented in addition to other parameters.

[0093] The genetic modification of the plant cells or of the plants is elicited by mutagenesis of one or more genes. The nature of the mutation is immaterial in this regard as long as it leads to a reduction in the GBSSI and/or SSIII and/or BEI activities(-ies) and to a reduction in the expression of the gene specified under SEQ ID NO 11 or SEQ ID NO 13.

[0094] In connection with the present invention, the term "mutagenesis" is to be understood as meaning any type of introduced mutations, such as deletions, point mutations (nucleotide substitutions), insertions, inversions, gene conversions or chromosome translocations.

[0095] A mutation which leads to a reduction in the GBSSI and/or SSIII and/or BEI activities(-ies) and to a reduction in the expression of the gene specified under SEQ ID NO 11 or SEQ ID NO 13 can arise spontaneously in a plant and the corresponding plants can be selected and propagated using the methods which are described below.

[0096] A mutation which leads to a reduction the GBSSI and/or SSIII and/or BEI activities(-ies) and to a reduction in the expression of the gene specified under SEQ ID NO 11 or SEQ ID NO 13 can also be produced by using chemical agents or energy-rich radiation (e.g. x-radiation, neutron radiation, gamma radiation or UV radiation).

[0097] Agents which can be used for producing chemically induced mutations, and the mutations which arise in this connection as a result of the action of the corresponding mutagens, are described, for example, by Ehrenberg and Husain (1981, Mutation Research 86, 1-113) and Müller (1972, Biologisches Zentralblatt 91 (1), 31-48). The generation of rice mutants using gamma rays, ethylmethane sulfonate (EMS), N-methyl-N-nitrosourea or sodium azide (NaN_3) is described, for example, by Jauhar and Siddiq (1999, Indian Journal of Genetics, 59 (1), 23-28), Rao (1977, Cytologica 42, 443-450), Gupta and Sharma (1990, Oryza 27, 217-219) and Satoh and Omura (1981, Japanese Journal of Breeding 31 (3), 316-326). The generation of wheat mutants using NaN_3 or maleic hydrazide is described by Arora et al. (1992, Annals of Biology 8 (1), 65-69). Scarascia-Mugnozza et al. (1993, Mutation Breeding Review 10, 1-28) provide a review of the generation of wheat mutants using different types of energy-rich radiation and chemical agents. Svec et al. (1998, Cereal Research Communications 26 (4), 391-396) describe the use of N-ethyl-N-nitrosourea for generating mutants

in triticale. The use of MMS (methylmethanesulfonic acid) and gamma radiation for generating millet mutants has been described by Shashidhara et al. (1990, Journal of Maharashtra Agricultural Universities 15 (1), 20-23).

[0098] The production of mutants in plant species which chiefly multiply vegetatively has been described, for example, in the case of potatoes which produce an altered starch (Hovenkamp-Hermelink et al., 1987, see above) and in the case of mint in which the oil yield is increased or the oil quality is altered (Dwivedi et al., 2000, Journal of Medicinal and Aromatic Plant Sciences 22, 460-463).

[0099] All these methods are in principle suitable for producing the plant cells or the plants.

[0100] Methods which are known to the skilled person can be used to find mutations in the corresponding genes, in particular in genes which encode GBSSI, SSIII or BEI or genes which exhibit the nucleotide sequence specified under SEQ ID NO 11 or SEQ ID NO 13. In particular, it is possible to make use, for this purpose, of analyses which are based on hybridizations with probes (southern blotting), amplification by means of the polymerase chain reaction (PCR), sequencing of relevant genomic nucleic acid fragments and searching for individual nucleotide substitutions. A method for identifying mutations with the aid of hybridization patterns is, for example, that of searching for restriction fragment length differences (restriction fragment length polymorphisms, RFLPs) (Nam et al., 1989, The Plant Cell 1, 699-705; Leister and Dean, 1993, The Plant Journal 4 (4), 745-750). An example of a PCR-based method is that of analyzing amplified fragment length differences (amplified fragment length polymorphisms, AFLPs) (Castiglioni et al., 1998, Genetics 149, 2039-2056; Meksem et al., 2001, Molecular Genetics and Genomics 265, 207-214; Meyer et al., 1998, Molecular and General Genetics 259, 150-160). It is also possible to use restriction endonuclease-cut amplified fragments (cleaved amplified polymorphic sequences, CAPS) for identifying mutations (Konieczny and Ausubel, 1993, The Plant Journal 4, 403-400; Jarvis et al., 1994, Plant Mol. Biol. 24, 685-687; Bachem et al., 1996, The Plant Journal 9 (5), 745-753). Methods for determining SNPs have been described by, inter alia, Qi et al. (2001, Nucleic Acids Research 29 (22), e116), Drenkard et al. (2000, Plant Physiology 124, 1483-1492) and Cho et al. (1999, Nature Genetics 23, 203-207). Methods which enable many plants to be examined within a short time for the presence of mutations in given genes are particularly suitable. Such a method, i.e. what is termed TILLING (targeting-induced local lesions in genomes) has been described by McCallum et al. (2000, Plant Physiology 123, 439-442).

[0101] All these methods are in principle suitable for identifying plant cells or the plants.

[0102] Hoogkamp et al. (2000, Potato Research 43, 179-189) have produced stable monoploid mutants from a potato mutant (*amf*) which was produced by means of chemical mutagenesis. These plants no longer synthesize any active GBSSI and therefore produce a starch which is amylose-free. The monoploid potato plants which are obtained can be used as the starting material for further mutageneses.

[0103] A reduction in the GBSSI and/or SSIII and/or BEI activities(-ies), and a reduction in the expression of the gene specified under SEQ ID NO 11 or SEQ ID No 13, can be achieved by reducing the expression of one or more of the gene(s) which encode(s) GBSSI or SSIII or BEI and which exhibit(s) the nucleotide sequence specified under SEQ ID NO 11 or SEQ ID NO 13 and/or by reducing the quantity of relevant enzyme material in the plant cells and/or by reducing the enzymic activity of the relevant proteins in the plant cells.

[0104] The reduction in the expression can, for example, be determined by measuring the quantity of transcripts which encode the relevant enzymes, e.g. by means of Northern blot analysis or RT-PCR. In this connection, a reduction preferably denotes a reduction in the quantity of transcripts by at least 50%, in particular by at least 70%, preferably by at least 85%, and particularly preferably by at least 95%, as compared with the quantity in corresponding wild-type plant cells.

[0105] The reduction in the quantity of GBSSI and/or SSIII and/or BEI, which results in a reduction in the relevant enzyme activities(-ies) in the plant cells, can be determined, for example, using immunological methods such as Western blot analysis, ELISA (enzyme-linked immunosorbent assay) or RIA (radioimmune assay). In this connection, a reduction preferably denotes a reduction in the quantity of relevant protein by at least 50%, in particular by at least 70%, preferably by at least 85%, and particularly preferably by at least 95%, as compared with that in corresponding wild-type plant cells.

[0106] The genetic modification of the plant cell comprises introducing one or more foreign nucleic acid molecules/polynucleotides whose presence and/or expression leads to a reduction in the GBSSI and/or SSIII and/or BEI activities(-ies), and to a reduction in the expression of the gene specified under SEQ ID NO 11 or SEQ ID NO 13, as compared with that/those in corresponding wild-type plant cells. In particular, the term genetic modification is understood as meaning the introduction of homologous and/or heterologous and/or mutagenized foreign nucleic acid molecules/polynucleotides into a plant cell, with said introduction of these molecules leading to a reduction in the GBSSI and/or SSIII and/or BEI activities(-ies) and to a reduction in the expression of the gene which is specified under SEQ ID NO 11 or SEQ ID NO 13. In this way, it is consequently possible to generate transgenic plant cells.

[0107] In this connection, the term "transgenic" means that the genetic information in the plant cells differs from that of corresponding wild-type plant cells as a result of the introduction of a foreign nucleic acid molecule/polynucleotide, or several foreign nucleic acid molecules/polynucleotides, into the cell.

[0108] In connection with the present invention, the term "foreign nucleic acid molecule/polynucleotide" or "foreign nucleic acid molecules/polynucleotides" is to be understood as meaning such a molecule which either naturally does

not occur in corresponding wild-type plant cells or which does not occur naturally in the specific spatial arrangement in corresponding wild-type plant cells, or which is located at a site in the genome of the plant cell at which it does not naturally occur. Preference is given to the foreign nucleic acid molecule/polynucleotide being a recombinant molecule which is composed of different elements whose combination, or specific spatial arrangement, does not occur naturally in plant cells.

[0109] The foreign nucleic acid molecule(s)/polynucleotide(s) which is/are used for the genetic modification can be one integrated nucleic acid construct or several separate nucleic acid constructs, in particular what are termed single, double, triple or quadruple constructs. Thus, the foreign nucleic acid molecule/polynucleotide can, for example, be what is termed a "quadruple construct", which is understood as meaning a single vector for plant transformation, which vector contains the genetic information for inhibiting the expression of one or more endogenous GBSSI genes, for inhibiting the expression of one or more SSIII genes, for inhibiting the expression of one or more BEI genes and for inhibiting the expression of the gene specified under SEQ ID NO 11 or SED ID NO 13, or whose presence or whose expression leads to a reduction in the GBSSI, SSIII and BEI activities and to a reduction in the expression of the gene specified under SEQ ID NO 11 or SEQ ID NO 13.

[0110] The foreign nucleic acid molecule/polynucleotide can be what is termed a "double construct", which is understood as meaning a vector for plant transformation which contains the genetic information for inhibiting the expression of two of the four target genes (GBSSI gene, SSIII gene, BEI gene, gene having the nucleotide sequence specified under SEQ ID NO 11 or SEQ ID NO 13) or whose presence or whose expression leads to a reduction in the activity of two of the four enzymes (GBSSI, SSIII, BEI or protein having the amino acid sequence specified under SEQ ID NO 12 or SEQ ID NO 14). In this example, the expression of the third and fourth target genes is inhibited, and/or the activities of the third and fourth enzymes are reduced, using a separate foreign nucleic acid molecule/polynucleotide which contains the appropriate genetic information for inhibiting these two additional target genes.

[0111] In another example, several different foreign nucleic acid molecules/polynucleotides, rather than a quadruple construct, are introduced into the genome of the plant cell, with one of these foreign nucleic acid molecules being, for example, a DNA molecule which, for example, constitutes a cosuppression construct which brings about a reduction in the expression of one or more endogenous GBSSI genes and leads to the inhibition of the expression of the gene specified under SEQ ID NO 11 or SEQ ID NO 13, and another foreign nucleic acid molecule being a DNA molecule which, for example, encodes an antisense RNA which beings about a reduction in the expression of one or more endogenous SSIII and/or BEI genes. In principle, however, the use of any combination of antisense, cosuppression, ribozyme and double-stranded RNA constructs or in-vivo mutagenesis which leads to a simultaneous reduction in the expression of endogenous genes which encode BGSSI, SSIII and BEI or which exhibit the nucleotide sequence specified under SEQ ID NO 11 or SEQ ID NO 13, or which leads to a simultaneous reduction in the GBSSI, SSIII or BEI activities and to inhibition of the expression of the gene specified under SEQ ID NO 11 or SEQ ID NO 13, is also suitable when constructing the foreign nucleic acid molecules.

[0112] In this connection, the foreign nucleic acid molecules can either be inserted into the genome of the plant cell simultaneously (cotransformation) or consecutively, that is chronologically one after the other (supertransformation).

[0113] The foreign nucleic acid molecules/polynucleotides can also be introduced into different individual plants belonging to a species. In this connection, it is possible to generate plants in which the activities(-ies) of one enzyme (e.g. GBSSI or SSIII or BEI) or of two enzymes (e.g. GBSSI and SSIII or GBSSI and BEI or SSIII and BEI) or of three enzymes is/are reduced. Subsequent crossing can then be used to generate plants in which the activities of all three enzymes (GBSSI, SSIII and BEI) are reduced and the expression of the gene specified under SEQ ID NO 11 or SEQ ID NO 13 is reduced.

[0114] It is furthermore possible, for the purpose of introducing a foreign nucleic acid molecule/polynucleotide, or for the purpose of producing the plant cells according to the invention or the plants according to the invention, to use a mutant instead of a wild-type plant cell or wild-type plant, with the mutant being distinguished by the fact that it already exhibits a reduced activity of one or more enzymes (GBSSI, SSIII, BEI and protein having the amino acid sequence specified under SEQ ID NO 12 or SEQ ID NO 14). The mutants can be either spontaneously arising mutants or else mutants which have been generated by the selective use of mutagens. Possibilities for generating such mutants have been described above.

[0115] The plant cells can also be produced by using what is termed insertion mutagenesis (review article: Thomeycroft et al., 2001, Journal of Experimental Botany 52 (361), 1593-1601). "Insertion mutagenesis" is to be understood, in particular, as being the insertion of transposons or of what is termed transfer DNA (T-DNA) into a gene which encodes GBSSI and/or SSIII and/or BEI and/or has the nucleotide sequence specified under SEQ ID NO 11 or SEQ NO 13.

[0116] The transposons can be either those which naturally occur in a wild-type plant cell (endogenous transposons) or those which do not naturally occur in said cell but which are introduced into the cell using genetic methods such as transformation of the cell (heterologous transposons). The skilled person is familiar with using transposons to change the expression of genes. Ramachandran and Sundaresan (2001, Plant Physiology and Biochemistry 39, 234-252) have provided a review regarding the use of endogenous and heterologous transposons as tools in plant biotechnology. The

possibility of identifying mutants in which specific genes have been inactivated by transposon insertion mutagenesis is described in a review by Maes et al. (1999, Trends in Plant Science 4 (3), 90-96). The use of endogenous transposons to generate rice mutants has been described by Hirochika (2001, Current Opinion in Plant Biology 4, 118-122). The use of endogenous retrotransposons to identify corn genes is described, for example, by Hanley et al. (2000, The Plant Journal 22 (4), 557-566). Kumar and Hirochika (2001, Trends in Plant Science 6 (3), 127-134) describe the possibility of using retrotransposons to produce mutants, and also describe methods for identifying mutants. The activities of heterologous transposons in different species have been described both in the case of dicotyledonous plants and in the case of monocotyledonous plants: e.g. in the case of rice (Greco et al., 2001, Plant Physiology 125, 1175-1177; Liu et al., 1999, Molecular and General Genetics 262, 413-420; Hiroyuki et al., 1999, The Plant Journal 19 (5), 605-613; Jeon and Gynheung, 2001, Plant Science 161, 211-219), barley (Koprek et al., 2000, The Plant Journal 24 (2), 253-263), Arabidopsis thaliana (Aarts et al., 1993, Nature 363, 715-717; Schmidt and Willmitzer, 1989, Molecular and General Genetics 220, 17-24; Altmann et al., 1992, Theoretical and Applied Genetics 84, 371-383; Tissier et al., 1999, The Plant Cell 11, 1841-1852), tomato (Belzile and Yoder, 1992, The Plant Journal 2 (2), 173-179) and potato (Frey et al., 1989, Molecular and General Genetics 217, 172-177; Knapp et al., 1988, Molecular and General Genetics 213, 285-290).

[0117] In principle, the plant cells and the plants can be produced using either homologous or heterologous transposons, with the use of homologous transposons also having to be understood as meaning the transposons which are already naturally present in the plant genome.

[0118] T-DNA insertion mutagenesis is based on specific segments (T-DNA) of Agrobacterium Ti plasmids being able to integrate into the genome of plant cells. The site for the integration into the plant chromosome is not fixed; rather, integration can take place at any arbitrary site. If the T-DNA integrates into a segment of the chromosome which constitutes a gene function, the integration can then lead to a change in the expression of the gene and consequently also to a change in the activity of a protein which is encoded by the gene in question. In particular, integration of a T-DNA into the coding region of a gene frequently results in the relevant cell either no longer being able to synthesize the corresponding protein at all or else no longer being able to synthesize it in an active form. The use of T-DNA insertions for generating mutants has been described, for example, in the case of Arabidopsis thaliana (Krysan et al., 1999, The Plant Cell 11, 2283-2290; Atipiroz-Leehan and Feldmann, 1997, Trends in Genetics 13 (4), 152-156; Parinov and Sundaresan, 2000, Current Opinion in Biotechnology 11, 157-161) and rice (Jeon and An, 2001, Plant Science 161, 211-219; Jeon et al., 2000, The Plant Journal 22 (6), 561-570). Methods for identifying mutants which have been generated using T-DNA insertion mutagenesis have been described, inter alia, by Young et al. (2001, Plant Physiology 125, 513-518), Parinov et al. (1999, The Plant Cell 11, 2263-2270), Thorneycroft et al. (2001, Journal of Experimental Botany 52, 1593-1601) and McKinney et al. (1995, The Plant Journal 8 (4), 613-622).

[0119] T-DNA mutagenesis is in principle suitable for generating the plant cells and the plants.

[0120] In another example, the presence and/or the expression of one or more foreign nucleic acid molecules/polynucleotides leads to the expression of endogenous genes which encode GBSSI and/or SSIII and/or BEI, and/or which exhibit the nucleotide sequence specified under SEQ ID NO 11 or SEQ ID NO 13, being inhibited.

[0121] This can be achieved by means of a variety of methods which are known to a skilled person. These methods include, for example, expressing a corresponding antisense RNA or a double-stranded RNA construct, providing molecules or vectors which mediate a cosuppression effect, expressing an appropriately constructed ribozyme which specifically cleaves transcripts which encode GBSSI or SSIII or BEI, or what is termed "in-vivo mutagenesis". Furthermore, a reduction in the GBSSI and/or SSIII and/or BEI activities(-ies) and/or a reduction in the expression of the gene specified under SEQ ID NO 11 or SEQ ID NO 13 in the plant cells can also be elicited by simultaneously expressing sense and antisense RNA molecules of the respective target gene to be repressed, preferably of the GBSSI and/or SSIII and/or BEI gene and/or of the gene having the nucleotide sequence which is specified under SEQ ID NO 11 or SEQ ID NO 13. The skilled person is familiar with these methods.

[0122] In addition to this, it is known that, in planta, the formation of double-stranded RNA molecules of promoter sequences can lead in trans to methylation and transcriptional inactivation of homologous copies of this promoter (Mette et al., 2000, EMBO J. 19, 5194-5201).

[0123] In order to employ antisense or cosuppression technology for inhibiting gene expression, it is possible, for example, to use a DNA molecule which comprises the entire sequence encoding GBSSI and/or SSIII and/or BEI and/or the nucleotide sequence which is specified under SEQ ID NO 11 or SEQ ID NO 13, including any flanking sequences which may possibly be present, or else use DNA molecules which only comprise parts of the coding sequence, with these parts having to be sufficiently long to bring about an antisense effect or cosuppression effect in the cells. In general, sequences having a minimum length of 15 bp, preferably having a minimum length of 20-30 bp, particularly preferably having a length of 100-500 bp, in particular sequences having a length of more than 500 bp, are suitable for exerting very efficient antisense or cosuppression inhibition.

[0124] The use of polynucleotide sequences which have a high degree of identity with the sequences which occur endogenously in the plant cell and which encode GBSSI or SSIII or BEI, or which are depicted under SEQ ID NO 11 or SEQ ID NO 13, is also suitable for antisense or cosuppression approaches. The minimum identity should be greater

than approx. 65%. The use of sequences having identifies of at least 90%, in particular of between 95 and 100%, is to be preferred.

[0125] It is furthermore also possible to conceive of using introns, i.e. noncoding regions of genes which encode GBSSI or SSIII or BEI or which exhibit the nucleotide sequence depicted under SEQ ID NO 11 or SEQ ID NO 13, for achieving an antisense or cosuppression effect.

[0126] The use of intron sequences for inhibiting the expression of genes which encode starch biosynthesis proteins has been described in WO 97/04112, WO 97/04113, WO 98/37213 and WO 98/37214.

[0127] The skilled person knows how he can achieve an antisense or cosuppression effect. The method of cosuppression inhibition has been described, for example, by Jorgensen (1990, Trends Biotechnol. 8, 340-344), Niebel et al. (1995, Top. Microbiol. Immunol. 197, 91-103), Flavell et al. (1995, Curr. Top. Microbiol. Immunol. 197, 43-46), Palauqui and Vaucheret (1995, Plant Mol. Biol. 29, 149-159), Vaucheret et al. (1995, Mol. Gen. Genet. 248, 311-317) and de Borne et al. (1994, Mol. Gen. Genet. 243,613-621).

[0128] Expression of ribozymes for the purpose of reducing the activity of particular enzymes in cells is also known to the skilled person and is described, for example, in EP-B1 0321201. The expression of ribozymes in plant cells has been described, for example, by Feyter et al. (1996, Mol. Gen. Genet. 250, 329-338).

[0129] Furthermore, a reduction in the GBSSI and/or SSIII and/or BEI activities(-ies) and/or a reduction in the expression of the gene depicted under SEQ ID NO 11 or SEQ ID NO 13 in the plant cells can also be achieved by what is termed "in-vivo mutagenesis", in which transformation of cells is used to introduce a hybrid RNA-DNA oligonucleotide ("chimeroplast") into cells (Kipp et al., poster session at the 5th International Congress of Plant Molecular Biology, 21-27 September 1997, Singapore; R.A. Dixon and C.J. Arntzen, meeting report relating to Metabolic Engineering in Transgenic Plants, Keystone Symposia, Copper Mountain, CO, USA, 1997, TIBTECH 15, 441-447; WO 95/15972; Kren et al., 1997, Hepatology 25, 1462-1468; Cole-Strauss et al., 1996, Science 273, 1386-1389 and Beetham et al., 1999, PNAS 96, 8774-8778).

[0130] While a part of the DNA component of the RNA-DNA oligonucleotide is homologous with a polynucleotide sequence in an endogenous GBSSI and/or SSIII and/or BEI gene and/or a gene depicted under SEQ ID NO 11 or SEQ ID NO 13, it exhibits a mutation as compared with the polynucleotide sequence of an endogenous GBSSI or SSIII or BEI gene or contains a heterologous region which is surrounded by the homolgous regions. As a result of base pairing of the homologous regions of the RNA-DNA oligonucleotide and of the endogenous polynucleotide, followed by homologous recombination, the mutation or heterologous region contained in the DNA component of the RNA-DNA oligonucleotide can be transferred into the genome of a plant cell. This leads to a reduction in the activities(-ies) of GBSSI and/or SSIII and/or BEI and/or to a reduction in the expression of the gene which is specified under SEQ ID NO 11 or SEQ ID NO 13.

[0131] In addition, a reduction in the GBSSI and/or SSIII and/or BEI activities(-ies) in the plant cells can also be elicited by simultaneously expressing sense and antisense RNA molecules of the respective target gene to be repressed, preferably of the GBSSI and/or SSIII and/or BEI gene and/or of the gene which is specified in SEQ ID NO 11 or SEQ ID NO 13.

[0132] This can be achieved, for example, by using chimeric constructs which contain inverted repeats of the respective target gene or parts of the target gene. In this connection, the chimeric constructs encode sense and antisense RNA molecules of the respective target gene. In planta, sense and antisense RNA are synthesized simultaneously as one RNA molecule, with sense and antisense RNA being separated from each other by a spacer and being able to form a double-stranded RNA molecule (RNAi technology).

[0133] It has been shown that introducing inverted-repeaT-DNA constructs into the genome of plants is a very efficient method for repressing the genes which correspond to the inverted-repeaT-DNA constructs (Waterhouse et al., 1998, Proc. Natl. Acad. Sci. USA 95, 13959-13964; Wang and Waterhouse, 2000, Plant Mol. Biol. 43, 67-82; Singh et al., 2000, Biochemical Society Transactions 28 (6), 925-927; Liu et al., 2000, Biochemical Society Transactions 28 (6), 927-929; Smith et al., 2000, Nature 407, 319-320; WO 99/53050). Sense and antisense sequences of the target gene or the target genes can also be expressed separately from each other using identical or different promoters (Nap et al., 6th International Congress of Plant Molecular Biology, 18-24 June 2000, Quebec, poster S7-27, lecture session S7).

[0134] It is consequently also possible to reduce the GBSSI and/or SSIII and/or BEI activities(-ies), and to reduce the expression of the gene specified under SEQ ID NO 11 or SEQ ID NO 13, in the plant cells by producing double-stranded RNA molecules of GBSSI or SSIII or BEI genes or genes having the nucleotide sequence which is depicted under SEQ ID NO 11 or SEQ ID NO 13. For this, preference is given to introducing inverted repeats of DNA molecules which are derived from GBSSI or SSIII or BEI genes, or genes having the nucleotide sequence depicted under SEQ ID NO 11 or SEQ ID NO 13, or cDNAs, into the genome of plants, with the DNA molecules which are to be transcribed being under the control of a promoter which regulates the expression of said DNA molecules.

[0135] In addition to this, it is known that, in plants, forming double-stranded RNA molecules of promoter DNA molecules can lead in trans to methylation and transcriptional inactivation of homolgous copies of these promoters, which will be termed target promoters in that which follows (Mette et al., 2000, EMBO J. 19, 5194-5201).

[0136] It is consequently possible to use inactivation of the target promoter to reduce the expression of a particular target gene (e.g. GBSSI, SSIII or BEI gene; gene having the nucleotide sequence depicted under SEQ ID NO 11 or SEQ ID NO 13) which is naturally under the control of this target promoter.

[0137] That is, in this case, the DNA molecules which comprise the target promoters of the genes (target genes) to be repressed are not, in contrast to the original function of promoters in plants, being used as elements for regulating the expression of genes or cDNAs but, instead, themselves being used as transcribable DNA molecules.

[0138] In order to generate the double-stranded target promoter RNA molecules in planta, where the molecules can be present as RNA hairpin molecules, preference is given to using constructs which contain inverted repeats of the target promoter DNA molecules, with the target promoter DNA molecules being under the control of a promoter which regulates the expression of said target promoter DNA molecules. These constructs are then introduced into the genome of plants. The expression of the inverted repeats of said target promoter DNA molecules leads, in planta, to the formation of double-stranded target promoter RNA molecules (Mette et al., EMBO J. 19, 5194-5201). These molecules can inactivate the target promoter.

[0139] Reduction of the GBSSI and/or SSIII and/or BEI activities(-ies), and inhibition of the expression of the gene specified under SEQ ID NO 11 or SEQ ID NO 13, in the plant cells can consequently also be achieved by generating double-stranded RNA molecules of promoter sequences of GBSSI or SSIII or BEI genes or of genes having the nucleotide sequence depicted under SEQ ID NO 11 or SEQ ID NO 13. For this, preference is given to introducing inverted repeats of promoter DNA molecules of GBSSI and/or SSIII and/or BEI promoters into the genome of plants, with the target promoter DNA molecules (GBSSI, SSIII or BEI promoter) to be transcribed being under the control of a promoter which regulates the expression of said target promoter DNA molecules.

[0140] The skilled person also knows that he can reduce the GBSSI and/or SSIII and/or BEI activities(-ies), and inhibit the expression of the gene specified under SEQ ID NO 11 or SEQ ID NO 13, by expressing nonfunctional derivatives, in particular transdominant mutants, of the enzymes and/or by expressing antagonists/inhibitors of the enzymes.

[0141] Antagonists/inhibitors of the enzymes can, for example, be antibodies, antibody fragments or molecules having similar binding properties. For example, a cytoplasmic scFv antibody has been used to modulate the activity of the phytochrome A protein in recombinantly altered tobacco plants (Owen, 1992, Bio/Technology 10, 790-794; Review: Franken et al., 1997, Current Opinion in Biotechnology 8, 411-416; Whitelam, 1996, Trends Plant Sci. 1, 268-272).

[0142] In a general manner, any promoter which is active in plant cells is suitable for expressing the foreign nucleic acid molecule/polynucleotide (the foreign nucleic acid molecules/polynucleotides). In this connection, the promoter can be selected such that the expression takes place constitutively in the plants according to the invention or only in one particular tissue, at a particular timepoint in the development of the plant or at a timepoint which is determined by external influences. The promoter can be homologous or heterologous in relation to the plant.

[0143] Examples of appropriate promoters for expressing nucleic acids/polynucleotides which reduce the activity of a target gene are the promoter of the cauliflower mosaic virus 35S RNA and the corn ubiquitin promoter for constitutive expression, the patatin gene promoter B33 (Rocha-Sosa et al., 1989, EMBO J. 8, 23-29), the MCPI promoter of the potato metallocarboxypeptidase inhibitor gene (HU 9801674) or the potato GBSSI promoter (WO 92/11376) for tuber-specific expression in potatoes or a promoter which allows expression only in photosynthetically active tissues, e.g. the ST-LS1 promoter (Stockhaus et al., 1987, Proc. Natl. Acad. Sci. USA 84, 7943-7947; Stockhaus et al., 1989, EMBO J. 8, 2445-2451), the Ca/b promoter (see, for example, US 5,656,496; US 5,639,952; Bansal et al., 1992, Proc. Natl. Acad. Sci. USA 89, 3654-3658) and the Rubisco SSU promoter (see, for example, US 5,034,322; US 4,962,028) or, for endosperm-specific expression, the glutelin promoter (Leisy et al., 1990, Plant Mol. Biol. 14, 41-50; Zheng et al., 1993, Plant J. 4, 357-366; Yoshihara et al., 1996, FEBS Lett. 383, 213-218), the shrunken-1 promoter (Werr et al., 1985, EMBO J. 4, 1373-1380), the wheat HMG promoter, the USP promoter, the phaseolin promoter or corn zein gene promoters (Pedersen et al., 1982, Cell 29, 1015-1026; Quatroccio et al., 1990, Plant Mol. Biol. 15, 81-93).

[0144] The potato patatin gene, MCPI and GBSSI promoters are promoters which are preferred for expressing the foreign nucleic acid molecule/polynucleotide (the foreign nucleic acid molecules/polynucleotides).

[0145] It is particularly advantageous to express the foreign nucleic acid molecule/polynucleotide (the foreign nucleic acid molecules/polynucleotides) in those organs in the plant which store starch. The examples of these organs are the tuber of the potato plant or the grains or endosperm of corn, wheat or rice plants. Preference is therefore given to using promoters which mediate expression in these organs.

[0146] However, it is also possible to use promoters which are only activated at a timepoint which is determined by external influences (see, for example, WO 93/07279). Promoters of heat shock proteins, which permit simple induction, may be of particular interest in this connection. It is furthermore possible to use seed-specific promoters, such as the Vicia faba USP promoter, which ensures seed-specific expression in Vicia faba and other plants (Fiedler et al., 1993, Plant Mol. Biol. 22, 669-679; Bäumlein et al., 1991, Mol. Gen. Genet. 225, 459-467), and also fruit-specific promoters, as described, for example, in WO 91/01373.

[0147] It is furthermore possible for a termination sequence, which serves the purpose of correctly terminating the transcription and of adding a poly A tail to the transcript, with the tail being attributed a function in stabilizing the transcripts,

to be present. These elements are described in the literature (see, for example, Gielen et al., 1989, EMBO J. 8, 23-29) and can be substituted as desired.

[0148] A large number of techniques are available for introducing DNA into a host plant cell. These techniques include transforming plant cells with T-DNA using Agrobacterium tumefaciens or Agrobacterium rhizogenes as transforming agent, fusing protoplasts, injecting, electroporating the DNA, introducing the DNA by means of a biolistic approach, and also other possibilities.

[0149] The use of the agrobacterium-mediated transformation of plant cells has been investigated intensively and described adequately in EP-A 0120516 and by Hoekema (1985, The Binary Plant Vector System Offsetdrukkerij Kanters B.V., Alblasserdam, Chapter V), Fraley et al. (Crit. Rev. Plant Sci. 4, 1-46) and An et al. (1985, EMBO J. 4, 277-287). For information regarding potato transformation, see, for example, Rocha-Sosa et al., 1989, EMBO J. 8, 29-33.

[0150] The use of agrobacterium transformation-based vectors for transforming monocotyledonous plants has also been described (Chan et al., 1993, Plant. Mol. Biol. 22, 491-506; Hiei et al., 1994, Plant J. 6, 271-282; Deng et al., 1990, Science in China 33, 28-34; Wilmink et al., 1992, Plant Cell Reports 11, 76-80; May et al., 1995, Bio/Technology 13, 486-492; Conner and Domisse, 1992, Int. J. Plant Sci. 153, 550-555; Ritchie et al, 1993, Transgenic Res. 2, 252-265). Transformation using the biolistic approach (Wan and Lemaux, 1994, Plant Physiol. 104, 37-48; Vasil et al., 1993, Bio/Technology 11, 1553-1558; Ritala et al., 1994, Plant Mol. Biol. 24, 317-325; Spencer et al., 1990, Theor. Appl. Genet. 79, 625-631), protoplast transformation, electroporation of partially permeabilized cells, and the use of glass fibers to introduce DNA, represent alternative systems for transforming monocotyledonous plants. The transformation of corn, in particular, has been described repeatedly in the literature (see, for example, WO 95/06128, EP-A 0513849, EP-A 0465875, EP-A 0292435; Fromm et al., 1990, Biotechnology 8, 833-844; Gordon-Kamm et al., 1990, Plant Cell 2, 603-618; Koziel et al., 1993, Biotechnology 11, 194-200; Moroc et al., 1990, Theor. Appl. Genet. 80, 721-726).

[0151] The successful transformation of other cereal types has also been described, for example in the case of barley (Wan and Lemaux, see above; Ritala et al., see above; Krens et al., 1982, Nature 296, 72-74) and in the case of wheat (Nehra et al., 1994, Plant J. 5, 285-297).

[0152] The present disclosure also relates to a plant cell which is genetically modified, with the genetic modification leading to reduction of the GBSSI, SSIII and BEI activities and to inhibition of the expression of the gene specified under SEQ ID NO 11 or SEQ NO 13 as compared with those of corresponding wild-type plant cells or wild-type plants, and which contains at least one foreign nucleic acid which is selected from the group consisting of

a) polynucleotides which encode at least one antisense RNA which leads to a reduction in the expression of at least one endogenous GBSSI gene and/or to a reduction in the expression of at least one endogenous SSIII gene and/or to a reduction in the expression of at least one endogenous BEI gene and/or to a reduction in the expression of at least one gene having the nucleotide sequence specified under SEQ ID NO 11 or SEQ ID NO 13;

b) polynucleotides which lead, by way of a cosuppression effect, to a reduction in the expression of at least one endogenous GBSSI gene and/or to a reduction in the expression of at least one endogenous SSIII gene and/or to a reduction in the expression of at least one endogenous BEI gene and/or to a reduction in the expression of at least one gene having the nucleotide sequence specified under SEQ ID NO 11 or SEQ ID NO 13;

c) polynucleotides which encode at least one ribozyme which specifically cleaves transcripts of at least one endogenous GBSSI gene and/or of at least one SSIII gene and/or of at least one BEI gene and/or of at least one gene having the nucleotide sequence specified under SEQ ID NO 11 or SEQ ID NO 13;

d) polynucleotides which are introduced by means of in-vivo mutagenesis and which lead to a mutation or an insertion in at least one endogenous GBSSI gene and/or to a mutation or an insertion in at least one endogenous SSIII gene and/or to a mutation or an insertion in at least one endogenous BEI gene and/or to a mutation or an insertion in at least one gene having the nucleotide sequence specified under SEQ ID NO 11 or SEQ ID NO 13, with the mutation or insertion leading to a reduction in the expression of said gene or to the synthesis of inactive GBSSI and/or of inactive SSIII and/or of inactive BEI and/or of an inactive protein having the amino acid sequence specified under SEQ ID NO 12 or SEQ ID NO 14;

e) polynucleotides which encode at least one antisense RNA and at least one sense RNA, with said antisense RNA and said sense RNA being able to form a double-stranded RNA molecule which leads to a reduction in the expression of at least one endogenous GBSSI gene and/or to a reduction in the expression of at least one endogenous SSIII gene and/or to a reduction in the expression of at least one endogenous BEI gene and/or to a reduction in the expression of at least one gene having the nucleotide sequence specified under SEQ ID NO 11 or SEQ ID NO 13;

f) polynucleotides which contain transposons, with the integration of the transposon sequences leading to a mutation or an insertion in at least one endogenous GBSSI gene and/or to a mutation or an insertion in at least one endogenous SSIII gene and/or to a mutation or an insertion in at least one endogenous BEI gene, and/or to a mutation or an insertion in at least one gene having the nucleotide sequence specified under SEQ ID NO 11 or SEQ ID NO 13, with the mutation or insertion leading to a reduction in the expression of said gene or to the synthesis of inactive GBSSI and/or of inactive SSIII and/or of inactive BEI and/or of an inactive protein having the amino acid sequence

specified under SEQ ID NO 12 or SEQ ID NO 14; and

g) T-DNA molecules which, by insertion in at least one endogenous GBSSI gene and/or by insertion in at least one endogenous SSIII gene and/or by insertion in at least one endogenous BEI gene and/or by insertion in at least one gene having the nucleotide sequence specified under SEQ ID NO 11 or SEQ ID NO 13, lead to a reduction in the expression of said gene or to the synthesis of inactive GBSSI and/or of inactive SSIII and/or of inactive BEI and/or of an inactive protein having the amino acid sequence specified under SEQ ID NO 12 or SEQ ID NO 14.

[0153] The present disclosure also relates to any type of material for propagating plants according to the invention.

[0154] The plant cells can be used for regenerating whole plants.

[0155] The present disclosure likewise relates to the plants which can be obtained by regenerating the plant cells.

[0156] The plants or the plant cells can belong to any arbitrary plant species, that is either to monocotyledonous or to dicotyledonous plants. The plants are preferably agriculturally useful plants, i.e. plants which are cultivated by man for purposes of nutrition or for technical, in particular, industrial purposes, and their cells. The disclosure fiber-forming (e.g. flax, hemp and cotton), oil-storing (e.g. rape, sunflower and soybean), sugar-storing (e.g. sugar beet, sugar cane and sweet sorghum) and protein-storing plants (e.g. leguminosae) and their cells.

[0157] The disclosure relates to forage plants, in particular forage grasses and pasture grasses (alfalfa, clover, etc.) and vegetable plants (e.g. tomato, salad and chicory) and their cells.

[0158] The disclosure relates to starch-storing plants (e.g. wheat, barley, oats, rye, potato, corn, rice, pea and tapioca), particularly preferably potatoes, and their cells.

[0159] In connection with the present invention, the term "potato plant" or "potato" means plant species of the genus *Solanum*, particularly tuber-producing species of the genus *Solanum* and, in particular, *Solanum tuberosum*.

[0160] The present disclosure furthermore relates to a method for producing a plant in which

a) a plant cell is genetically modified, with the genetic modification being the introduction of one or more foreign nucleic acid molecule(s) whose presence and/or expression leads to a reduction in the GBSSI and/or SSIII and/or BEI activities(-ies) and to a reduction in the expression of the gene specified under SEQ ID NO 11 or SEQ ID NO 13, as compared with those in corresponding wild-type plant cells,

b) a plant is regenerated from step a) plant cells; and

c) where appropriate, further plants are produced using the plants in accordance with step b).

[0161] The genetic modification which is introduced into the plant cell in accordance with step a) can in principle be any type of modification which leads to a reduction in the activity of one or more SSIII proteins which occur(s) endogenously in the plant and of one or more BEI proteins which occur(s) endogenously in the plant and of one or more GBSSI proteins which occur(s) endogenously in the plant and of one or more proteins which occur(s) endogenously in the plant and which exhibit(s) at least 80%, preferably 90%, particularly preferably 95%, identity with the nucleic acid molecule specified under SEQ ID NO 12 or SEQ ID NO 14.

[0162] The plants according to step (b) can be regenerated using methods which are known to the skilled person (e.g. described in "Plant Cell Culture Protocols", 1999, edtd. by R.D. Hall, Humana Press, ISBN 0-89603-549-2). Further plants can be regenerated in accordance with step (c) of the method by means, for example, of vegetative propagation (for example using cuttings or tubers or using a callus culture and regenerating whole plants) or by means of sexual propagation. In this connection, the sexual propagation preferably takes place in a controlled manner, i.e. selected plants possessing particular properties are crossed with each other and propagated. In this connection, the selection is preferably made such that the further plants which are obtained in accordance with step c) exhibit the genetic modification which was introduced in step a).

[0163] It is referred to the disclosure content of all the documents cited in the patent application and the present description of the invention.

General methods

[0164] The following methods were used in the examples. These methods are also intended to be employed in connection with the present invention:

1. Method for extracting starch from potatoes

[0165] All the tubers belonging to a line (from 4 to 5 kg) are worked up jointly in a commercially available juice extractor (Multipress automatic MP80, Braun). The starch-containing juice is collected in a 10 l bucket (ratio of the height of the bucket/diameter of the bucket = approx. 1.1) in which 200 ml of mains water containing a spoon tip (approx. 3-4 g) of sodium disulfite have been initially introduced. The bucket is then completely filled with mains water. After the starch

has settled for 2 hours the supernatant is decanted off and the starch is once again suspended in 10 l of mains water and passed through a sieve having a mesh width of 125 μm. After 2 hours (the starch has once again settled at the bottom of the bucket), the aqueous supernatant is decanted once again. This washing procedure is repeated a further 3 times such that the starch is resuspended in fresh mains water a total of five times. The starches are then dried at 37°C down to a water content of 12-17% and homogenized in a mortar. The starches are now available for analyses.

2. Starch analysis

a) Determining the amylose/amylopectin ratio

[0166] Starch was isolated from potato plants, as described above, and the amylose to amylopectin ratio was determined using the method described by Hovenkamp-Hermelink et al. (1988, Potato Research 31, 241-246). The amylose content is calculated by applying the formula cited on page 243 of this article.

b) Determining the phosphate content in the C6 position

[0167] In the starch, the C2, C3 and C6 positions of the glucose units can be phosphorylated. In order to determine the C6-P content of the starch, 50 mg of starch were hydrolyzed, at 95°C for 4 h, in 500 μl of 0.7 M HCl. The mixtures were then centrifuged at 15500 g for 10 min and the supernatants were taken off. 7 μl volumes of the supernatants were mixed with 193 μl of imidazole buffer (100 mM imidazole, pH 6.9; 5 mM $MgCl_2$, 1 mM EDTA and 0.4 mM NAD). The measurement was carried out at 340 nm in a photometer. After a basal absorption had been established, the enzyme reaction was started by adding 2U of glucose-6-phosphate dehydrogenase (from Leuconostoc mesenteroides, Boehringer Mannheim). The change in absorption is directly proportional to the concentration of the G-6-P content in the starch.

c) Using a Rapid Visco Analyzer (RVA) to determine the viscosity properties:

[0168] The viscosity properties were determined following the method which is described in WO 01/19975.

[0169] 2 g of starch (dry matter) were taken up in 25 ml of H_2O (deionized water, conductivity of at least 15 megaohm) and analyzed in a Rapid Visco Analyser (Newport Scientific Pty Ltd., Investmet Support Group, Warriewod NSW 2102, Australia) for the purpose of determining the viscosity properties. The appliance was operated in accordance with the manufacturer's instructions. In order to determine the viscosity of the aqueous solution of the starch, the starch suspension was first of all stirred at 960 rpm (revolutions per minute) for 10 seconds after which it was heated at 50°C for 1 min at a stirring rate of 160 rpm (step 1). After that, the temperature was increased from 50°C to 95°C at a heating rate of 12°C per min while the stirring rate remained the same (step 2). The temperature was then kept at 95°C for 2.5 min while the stirring rate remained the same (step 3). After that, the solution was cooled down from 95°C to 50°C at a cooling rate of 12°C per min while the stirring rate remained the same (step 4). The last step (step 5) maintains the temperature of 50°C for 2 min while the stirring rate remains the same. The viscosity was determined during the entire period.

[0170] After the program had come to an end, the stirrer was removed and the beaker was covered. The pasted starch was now available for the texture analysis after 24 h (method d) below).

[0171] In the RVA analysis profile, there are characteristic values which are presented for comparing different measurements and substances. In connection with the present invention, the following terms are to be understood as follows:

Maximum viscosity (RVA Max):

The maximum viscosity is understood as meaning the highest viscosity value, as measured in cP (centipoise), which is achieved in step 2 or 3 of the temperature profile.

Minimum viscosity (RVA Min):

The minimum viscosity is understood as meaning the lowest viscosity value, as measured in cP, which occurs in the temperature profile after the maximum viscosity. This normally occurs in step 3 of the temperature profile.

Final viscosity (RVA Fin):

The final viscosity is understood as meaning the viscosity value, as measured in cP, which occurs at the end of the measurement.

Setback (RVA Set):

What is termed the "setback" is calculated by subtracting the minimum viscosity value from the final viscosity.

Pasting temperature (RVA PT):

The pasting temperature is understood as being the temperature in the temperature profile at which the viscosity for the first time increases by 55 cP over a period of 20 sec.

d) Determining the gel strength (texture analyzer)

[0172] 2 g of starch (dry matter) were pasted in 25 ml of an aqueous suspension in the RVA appliance (temperature program: see under c) "using a Rapid Visco Analyzer (RVA) to determine the viscosity properties") and then stored, for 24 h at room temperature, in a closed vessel.

[0173] The samples were fixed under the probe (cylindrical plunger having a planar surface) of a TA-XT2 texture analyzer supplied by Stable Micro Systems (Surrey, UK), and the gel strength was determined using the following parameters:

- Test speed 0.5 mm/sec
- Penetration depth 7 mm
- Contact area 113 mm^2
- Pressure 2 g

e) Using ion exchange chromatography to analyze the side chain distribution of the amylopectin

[0174] In order to separate amylose and amylopectin, 200 mg of starch were dissolved in 50 ml reaction vessels containing 12 ml of 90% (v/v) DMSO in H_2O. After 3 volumes of ethanol had been added, the precipitate was separated off by centrifuging for 10 minutes at about 1800 g and at room temperature (RT). The pellet was then washed with 30 ml of ethanol, dried and dissolved at 75°C in 40 ml of 1% (w/v) NaCl solution. After the solution had been cooled down to 30°C, about 90 mg of thymol were added slowly and this solution was incubated at 30°C for at least 60 h. The solution was then centrifuged at 2000 g (RT) for 30 min. The supernatant was treated with 3 volumes of ethanol and the amylopectin which precipitated out was separated off by centrifuging at 2000 g (RT) for 5 minutes. The pellet (amylopectin) was then washed with ethanol and dried using acetone. A 1% solution of amylopectin was then prepared in 10 mM sodium acetate, pH 3.5, with the amylopectin being dissolved at 65-95°C for 1-2 h. In each case 100 µl of this solution were treated, for the digestion, with 180 µl of 10 mM sodium acetate, pH 3.5, and 1 µl of isoamylase (Megazyme) and the mixture was incubated at 37°C for about 16 h. A 1:5 aqueous dilution of this digestion was then filtered using an 0.2µm filter and 100µl of the filtrate were analyzed by ion chromatography (HPAEC-PAD, Dionex). The separation was effected using a PA-100 column (with appropriate precolumn), and the detection was effected amperometrically.

[0175] The elution conditions were as follows:

Solution A - 0.15 M NaOH

Solution B - 1 M sodium acetate in 0.15 M NaOH

t (min)	Solution A (%)	Solution B (%)
5	0	100
35	30	70
45	32	68
60	100	0
70	100	0
72	0	100
80	0	100
stop		

[0176] The relative proportion of short side chains in the total content of all the side chains was determined by determining the content of a particular side chain as a percentage of the total content of all the side chains. The total content of all the detectable side chains was elucidated by determining the total area under the peaks which represent the DP6 to 34 degrees of polymerization in the HPLC chromatogram.

[0177] The content of a particular side chain expressed as a percentage of the total content of all the side chains was elucidated by determining the ratio of the area of the peak which represents this side chain in the HPLC chromatogram to the total area. Version 6.20 of the Chromelion 6.20 program supplied by Dionex, USA, was used for determining the peak areas.

f) Using gel permeation chromatography to analyze the side chain distribution in total starch

[0178] In order to use gel permeation chromatography to determine the side chain distribution in total starch, 10 mg of starch were dissolved, at 60°C for approx. 3 h, in 250 μl of 90% (v/v) DMSO. After 375 μl of H_2O (dist.) had been added, the solution was heated at 95°C for approx. 1 h.

[0179] For the enzymic digestion of the starch, 200 μl of starch solution were added to 300 μl of 16.6 mM sodium acetate, pH 3.5, and incubated at 37°C for about 16 h by adding 2 μl of isoamylase (Megazyme). An aqueous 1:4 dilution of this digestion was then filtered using an 0.2 μm filter and 25 μl of the filtrate were analyzed by means of gel permeation chromatography. The separation was effected using two columns which were connected in series; that is firstly a Gram 3000 column (Polymer Standards Service with appropriate precolumn), with this then being followed by a Gram 100 column. A refraction index detector (RI 71, Shodex) was used for the detection. The column was equilibrated with 90% (v/v) DMSO, 90 mM sodium acetate. The column was eluted with 90% (v/v) DMSO, 90 mM sodium acetate at a flow rate of 0.7 ml/min and over a period of 1 h.

[0180] In order to correlate the elution volume with the molar mass, the columns which were used were calibrated with dextran standards (Fluka, product #31430). The dextrans which were used, their appurtenant molar masses, and the elution volumes, are shown in Table 1. The resulting calibration straight lines were used to depict the elution plot as a molecular weight distribution (Figure 1):

Tab. 1: Dextran standard calibration table

Elution volume (ml)	Molar mass [D]	Sample name
19.22	401300	dextran T670P
20.05	276500	dextran T410P
21.03	196300	dextran T270P
21.93	123600	dextran T150P
22.98	66700	dextran T80
24.00	43500	dextran T50
25.43	21400	dextran T25
27.22	9890	dextran T12
28.55	4440	dextran T5
30.92	1080	dextran T1

[0181] In this connection, the total area of the GPC chromatogram was divided into individual sections which represented respective groups of side chains of differing lengths. The sections which were chosen contain side chains having the following degrees of polymerization (dp = number of glucose monomers within a side chain): dp<12, dp12-19, dp20-25, dp26-31, dp32-37, dp38-43, dp44-49, dp50-56, dp57-62, dp63-123 and >dp123. The dextrans which were used, their appurtenant molar masses, and the elution volumes, are shown in Tab. 1. The resulting calibration straight lines are used to depict the elution plot as a molecular weight distribution (Fig. 1). In order to determine the molecular weights of the individual side chains, glucose was specified to have a molecular weight of 162. The total area in the GPC chromatogram is stipulated to be 100% and the amounts represented by the area of the individual sections are calculated based on the amount represented by the total area:

g) Determining the freeze/thaw stability

[0182] In order to determine the freeze/thaw stability, in each case 3.5 g of starch (dry weight) were made up to 70 ml with distilled water and pasted for 15 min at 90°C (128 rpm, inclined blade stirrer) in a rotary viscometer (Rotovisko, Haake). The starch paste was then autoclaved, at 121°C for 15 min, in a glass vessel having a screw closure. After that, in each case 5 g of this paste were subjected 3 times, likewise in a glass vessel having a screw closure, to a freeze/thaw cycle (from room temperature down to -20°C). This quantity of paste was then treated with 25 ml of distilled water, homogenized at 8000 rpm for 1 min in an Ultra-Turrax, and then extracted on a magnetic stirrer for 1 h at 37°C in a drying oven. The sample was then transferred to a 50 ml volumetric flask and made up to 50 ml with distilled water; this mixture was then centrifuged at 2800 g for 5 min and filtered. An aliquot of this filtrate was inspissated overnight at 105°C and the residue was weighed. The freeze/thaw stability was then calculated as follows:

$$\text{Freeze/thaw stability}(\%) = \frac{50 \times 100 \times \text{TS in the weighing pan (g)}}{\text{aliquot (g)} \times \text{starch TS in the sample (g)}}$$

h) Determining the heat stability

[0183] In order to determine the heat stability, in each case 3.5 g of starch (dry weight) were made up to 70 ml with distilled water and pasted for 15 min at 90°C (128 rpm, inclined blade stirrer) in a rotary viscometer (Rotovisko, Haake). The starch paste was then autoclaved, at 121 °C for 15 min, in a glass vessel having a screw closure and subsequently transferred back to the rotary viscometer beaker.

[0184] After 6 min at 90°C, and with the blade stirrer rotating at 128 rpm, the scale graduation was read and compared with the value which was measured after 21 min at 128 rpm and 90°C without any autoclaving.

$$\text{Heat stability (\%)} = \frac{\text{Scale graduation (after autoclaving)} \times 100}{\text{Scale graduation (21 min, 128 rpm, 90°C)}}$$

i) Differential scanning calorimetry (DSC) measurement

[0185] For a DSC measurement, 10 mg of starch were weighed into a stainless steel cup (volume 50 µl) containing 30 µl of distilled water. An empty stainless steel cup was used as reference. The sample was heated from 20°C to 120°C at a heating rate of 10°C/min in a Diamond DSC appliance (Perkin Elmer). The data were analyzed using a Pyres software program. This involved determining T(onset), T(peak) and the free enthalpy.

j) The total phosphate content was determined using the Ames method (Methods in Enzymology VIII, (1966), 115-118)

[0186] 30 µl of ethanolic magnesium nitrate solution are added to approx. 50 mg of starch and the mixture is incinerated at 660°C for three hours in a muffle furnace. 500 µl of 0.5 M hydrochloric acid are added to the residue and the mixture is incubated at 60°C for 30 min. An aliquot of 10 or 20 µl (depending on the expected phosphate content) is then made up to 300 µl with 0.5 M hydrochloric acid and this mixture is added to a mixture of 100 µl of 10% ascorbic acid and 600 µl of 0.42% ammonium molybdate in 0.5 M sulfuric acid and the whole is incubated at 45°C for 20 min.

[0187] This is then followed by a photometric determination at 820 nm using a phosphate calibration series as standard.

k) Determination of the shear stability of starches

[0188] Shear stability was determined using a "Viskotester VT 550" (Type 002 - 7026), Gebrüder Haake, Dieselstraße 4, D 76227 Karlsruhe.

[0189] 70 g of a starch suspension (5% w/v) were transferred to the container that is used for the measurements. This container was transferred to the heating device which had been adjusted to 90°C prior to that. Upon starting the measurement the slurry was stirred by a paddle agitator at 128 rpm (revolutions per minute) while the viscosity was recorded (software version 2.30.P). After 15 minutes the speed of the stirrer was increased to 512 rpm. After 5 minutes stirrer speed again was reduced to 128 rpm. Total time for the measurement was 21 minutes.

[0190] To determine the stability against shear forces, a suspension of the same starch having the same concentration was examined under identical conditions. However, in this second experiment the stirrer speed was kept at 128 rpm for the entire period of 21 min.

[0191] Viscosity is expressed as a relative viscosity having the unit "Skalenteile" (SKT).

[0192] Shear stability is expressed as the ratio of the viscosities after 21 min of the starch slurry that was stirred at 512 rpm to the slurry that was stirred at 128 rpm only.

$$\text{Shear stability (\%)} = \frac{\text{Viscosity}_{(21\ \text{min})}\ \text{stirred at 512 rpm} \times 100}{\text{Viscosity}_{(21\ \text{min})}\ \text{stirred at 128 rpm}}$$

I) Determination of the peak viscosity by the Rotovisko method

[0193] Peak viscosity was determined using a "Viskotester VT 550" (Type 002-7026) Gebrüder Haake, Dieselstraße 4, D 76227 Karlsruhe.

[0194] 70 g of a starch suspension (5% w/v) were transferred to the container that is used for the measurements. This container was transferred to the heating device which had been adjusted to 90°C prior to that. Upon starting the measurement the slurry was stirred by a paddle agitator at 128 rpm (revolutions per minute) while the viscosity was recorded (software version 2.30.P). The measurement was terminated after 15 minutes.

[0195] Viscosity is expressed as a relative viscosity having the unit "Skalenteile" (SKT).

[0196] Peak viscosity is the maximum viscosity as recorded during the period of measurement.

Description of the sequences

[0197]

Seq ID 1: Nucleic acid sequence of the potato *(Solanum tuberosum)* starch synthase SSIII, with the sequences which encode the corresponding SSIII protein being indicated.

Seq ID 2: Amino acid sequence of a potato SSIII protein.

Seq ID 3: Amino acid sequence of the Pfam cbm25 binding domain of the potato (*Solanum tuberosum*) SSIII protein.

Seq ID 4: Nucleic acid sequence encoding the potato (*Solanum tuberosum*) branching enzyme BEI.

Seq ID 5: Amino acid sequence of the potato *(Solanum tuberosum)* branching enzyme BEI.

Seq ID 6: Coding nucleic acid sequence of the potato (*Solanum tuberosum*) GBSSI gene.

Seq ID 7: Amino acid sequence of potato (*Solanum tuberosum*) GBSSI.

Seq ID 8: Primer B1_Asp

SEQ ID NO 9: Nucleic acid sequence containing the region encoding the 3' region of a *Solanum tuberosum* (cv Désirée) protein involved in starch biosynthesis. This sequence is inserted in plasmid AN 46-196.

SEQ ID NO 10: Nucleic acid sequence containing the region encoding the 5' region of a *Solanum tuberosum* (cv Désirée) protein involved in starch biosynthesis. This sequence is inserted in plasmid AN 47-196.

SEQ ID NO 11: Nucleic acid sequence containing the complete region encoding a *Solanum tuberosum* (cv Désirée) protein involved in starch biosynthesis. This sequence is inserted in plasmid AN 49. This plasmid was deposited, in accordance with the Budapest Treaty, on September 15, 2003 in the Deutsche Sammlung von Mikroorganismen und Zellkulturen [German Collection of Microorganisms and Cell Cultures] GmbH, Mascheroder Weg 1b, 38124 Braunschweig, Germany, under the number DSM 15926.

SEQ ID NO 12: Amino acid sequence encoding a *Solanum tuberosum* (cv Désirée) protein involved in starch biosynthesis. This sequence can be deduced from the nucleic acid sequence inserted in plasmid AN 49 or from the nucleic acid sequence described under SEQ ID NO 11.

SEQ ID NO 13: Nucleic acid sequence containing the complete region encoding a *Solanum tuberosum* (cv Désirée)

protein involved in starch biosynthesis. This sequence was obtained by joining together the nucleic acid sequences which are described under SEQ ID NO 9 and SEQ ID NO 10. This nucleic acid sequence constitutes an allelic variant of the nucleic acid sequence described under SEQ ID NO 11 encoding a protein involved in starch biosynthesis.

SEQ ID NO 14: Amino acid sequence encoding a *Solanum tuberosum* (cv Désirée) protein involved in starch biosynthesis. This sequence can be deduced from the nucleic acid sequence described under SEQ ID NO 13 and constitutes the amino acid sequence of an allelic variant of the amino acid sequence described under SEQ ID NO 12 encoding a protein involved in starch biosynthesis.

SEQ ID NO 15: Primer B2_Sal (TCA AGT CGA CCA CAA CCA GTC CAT TTC TGG)

SEQ ID NO 16 Primer KM2_Spe (5'-TCAAACTAGTCACAACCAGTCC-ATTTCTGG-3')

SEQ ID NO 17 Primer SoputE (5'-CACTTTAGAAGGTATCAGAGC-3')

SEQ ID NO 18 Primer So_put5' (5'-GTATTTCTGCGAAGGAACGACC-3')

SEQ ID NO 19 Primer So_putA (5'-AACAATGCTCTCTCTGTCGG-3')

SEQ ID NO 20 Primer B3_Sal (GCT TGT CGA CGG GAG AAT TTT GTC CAG AGG)

SEQ ID NO 21 Primer B4_Sal (GAT CGT CGA CAG CAC TTC TAC TTG GCA GAG G)

Description of the figures

[0198] Fig. 1: Calibration curve for GPC

Example 1

Producing transgenic potato plants which exhibit a reduced expression of the BEI, SSIII and GBSSI genes

[0199] In order to generate transgenic plants which exhibit reduced BEI, SSIII and GBSSI activities, transgenic plants which exhibited reduced BEI and SSIII activities were first of all generated. For this purpose, agrobacteria were used, as described in Rocha-Sosa et al. (1989, EMBO J. 8, 23-29), to transfer the T-DNA of the plasmid pB33-aBEI-aSSIII-Kan into potato plants.

[0200] In order to construct the plasmid pB33-aBEI -aSSIII-Kan, the expression vector pBin33-Kan was first of all constructed. For this, the promoter of the *Solanum tuberosum* patatin gene B33 (Rocha-Sosa et al., 1989, see above) was ligated, as a *Dra*I fragment (nucleotides -1512 - +14), into the vector pUC19 (Genbank Acc. No. M77789), which had been cut with *Sst*I and whose ends had been smoothed using T4 DNA polymerase. This resulted in the plasmid pUC19-B33. The B33 promoter was excised from this plasmid using *Eco*RI and *Sma*I and ligated into the vector pBinAR, which had been cut correspondingly. This resulted in the plant expression vector pBin33-Kan. The plasmid pBinAR is a derivative of the vector plasmid pBin19 (Bevan, 1984, Nucl. Acid Research 12, 8711-8721) and was constructed by Höfgen and Willmitzer (Plant Sci. 66 (1990), 221-230). A HindII fragment of 1631 bp in length, which contains a partial

cDNA encoding the potato BEI enzyme (Kossmann et al., 1991, Mol. Gen. Genet. 230(1-2):39-44), was then smoothed and introduced into vector pBinB33, which had been previously cut with *Sma*I, in the antisense orientation in regard to the B33 promoter (promoter of the *Solanum tuberosum* patatin gene B33; Rocha-Sosa et al., 1989, loc. cit.). The resulting plasmid was cut with *BamH*I. A *BamH*I fragment of 1363 bp in length, containing a partial cDNA encoding the potato SSIII protein (Abel et al., 1996, loc. cit.), was introduced into the cleavage site, likewise in the antisense orientation with regard to the B33 promoter.

[0201] In order to detect the activity of soluble starch synthases by means of nondenaturing gel electrophoresis, tissue samples of potato tubers were disrupted in 50 mM Tris-HCl, pH 7.6, 2 mM DTT, 2.5 mM EDTA, 10% glycerol and 0.4 mM PMSF. The electrophoresis was carried out in a MiniProtean II chamber (BioRAD). The monomer concentration of the gels, which were 1.5 mm thick, was 7.5% (w/v), while 25 mM Tris-glycine, pH 8.4, served as the gel buffer and running buffer. Equal quantities of protein extract were loaded on and fractionated for 2 h at 10 mA per gel.

[0202] The activity gels were then incubated in 50 mM Tricine-NaOH, pH 8.5, 25 mM potassium acetate, 2 mM EDTA, 2 mM DTT, 1 mM ADP-glucose, 0.1% (w/v) amylopectin and 0.5 M sodium citrate. Glucans which were formed were stained with Lugol's solution.

[0203] BEI activity was likewise detected using nondenaturing gel electrophoresis: in order to isolate proteins from plants, the sample material was triturated in liquid nitrogen, taken up in extraction buffer (50 mM Na citrate, pH 6.5; 1 mM EDTA, 4 mM DTT) and, after centrifugation (10 min, 14 000 g, 4°C), used directly for measuring the protein concentration as described by Bradford. From 5 to 20 μg, as required, of total protein extract were then treated with 4-fold loading buffer (20% glycerol, 125 mM Tris HCl, pH 6.8) and loaded onto a "BE activity gel". The composition of the running buffer (RB) was as follows: RB = 30.2 g of Tris base, pH 8.0, 144 g of glycine made up to 1 l with H_2O.

[0204] After the gel run had come to an end, the gels were in each case incubated overnight at 37°C in 25 ml of "phosphorylase buffer" (25 ml of 1 M Na citrate, pH 7.0, 0.47 g of glucose-1-phosphate, 12.5 mg of AMP, 2.5 mg of rabbit phosphorylase a/b). The gels were stained with Lugol's solution.

[0205] It was possible to identify different lines of transgenic potato plants whose tubers exhibited markedly reduced BEI and SSIII activities. The line (asBEI-asSSIII), whose isolated starches exhibited the highest phosphate content of all the independent transformants which were investigated, was then transformed with the plasmid p35SaGBSSI-Met as described in Rocha-Sosa et al. (1989, EMBO J. 8, 23-29).

[0206] Plasmid p35SaGBSSI-Met was prepared by inserting an Asp718/Xbal fragment of 1921 bp in length, containing a partial cDNA encoding potato GBSSI (Hergersberg, 1988, see above) into the vector pBinAR-Met, which had also been opened, in the antisense orientation with regard to the 35S promoter.

[0207] Vector pBinAR-Met is derived from the plasmid pGPTV-DHFR, which is a derivative of the vector pBin19 (Becker et al., 1992, Plant Mol. Biol. 20, 1195-1197). pBinAR-Met contains the dhfr gene, which mediates resistance to methotrexate, instead of the nptII gene and the 3' end of gene 7 of the T-DNA of the Ti plasmid pTiACH5 (nucleotides 2106-2316; Gielen et al., 1984, EMBO J. 3, 835-846) instead of the 3' end of the nopaline synthase gene. Taking plasmid pA7 (compare description of vector pBinAR above) as the starting point, the EcoRI/HindIII fragment, comprising the 35S promoter, the ocs terminator and the intercollated part of the polylinker, was ligated into plasmid pGPTV-DHFR, which was cut correspondingly. The resulting vector was designated pBinAR-Met.

[0208] Tissue samples were taken from tubers derived from the independent transformants among the plants which were obtained by transformation with the plasmid p35SaGBSSI-Met, and which were designated asBEI-asSSIII-asGBSSI plants, and the samples were stained with iodine solution and examined under the microscope. The starches of the independent lines whose granules stained brown were used for further analysis of the starch properties.

Example 2

Cloning a full-length sequence of a *Solanum tuberosum* gene having the sequence specified under SEQ ID NO 11 or 13

[0209] The nucleotide sequence (SEQ ID No. 11 or SEQ ID No.13) encoding a *Solanum tuberosum* protein having the amino acid sequence specified under SEQ ID No. 12 or SEQ ID No.14 has not been previously described. By making sequence comparisons using different branching enzymes, it was possible to identify a domain which was used to screen EST databases. The potato EST TC73137 (TIGR database; http://www.tigr.org/tigr-scripts/tgi/tc_report.pl?tc=TC73137&species=potato) was identified in this connection.

[0210] The primers B1_Asp (GAT GGG TAC CAG CAC TTC TAC TTG GCA GAG G = SEQ ID No. 8) and B2_Sal (TCA AGT CGA CCA CAA CCA GTC CAT TTC TGG = SEQ ID No. 15) were used to amplify a sequence, which corresponded to this EST sequence, from a tuber-specific *Solanum tuberosum* (cv Désirée) cDNA library. Attempts to use leaf-specific, sink or source tissue-specific cDNA libraries as templates for the PCR reaction did not give rise to any amplificate.

[0211] Primers which were complementary to the ends of the previously known sequence and vector sequences of

the relevant cDNA libraries were prepared for the purpose of amplifying the entire sequence encoding the protein concerned, which sequence also comprised previously unknown sequences. None of the primer combinations for amplifying a full-length sequence which were used when taking this approach led to any further region being amplified. Tomato EST databases were consequently screened once again.

[0212] In this connection, it was possible to identify two tomato ESTs (TIGR database; BG127920 and TC130382) which either exhibited a high degree of homology with the above-described amplificate of the potato protein (TC130382) or (BG127920) or with a putative branching enzyme derived from *Arabidopsis* (Genbank: GP|9294564|dbj|BAB02827.1).

[0213] Primers were now prepared once again in order to also amplify previously unknown sequences of the protein having the amino acid sequence depicted under SEQ ID NO 12 or SEQ ID NO 14. The 3' region of the protein concerned was amplified by means of PCR, using the primers KM2_Spe (5'-TCAAACTAGTCACAACCAGTCCATTTCTGG-3' = SEQ ID No. 16) and SoputE (5'-CACTTTAGAAGGTATCAGAGC-3' = SEQ ID No. 17), from a cDNA library which was prepared from *Solanum tuberosum* (cv Désirée) tubers. The resulting fragment, of approx. 1 kb in size, was cloned in an undirected manner into the pCR4-TOPO vector supplied by Invitrogen (product number: 45-0030). The resulting plasmid was designated AN 46-196. The sequence of the fragment inserted in plasmid AN 46-196 is depicted under SEQ ID NO 9.

[0214] The 5' region was likewise amplified by means of the PCR technique from the same cDNA library using the primers So_put5' (5'-GTATTTCTGCGAAGGAACGACC-3' = SEQ ID No. 18) and So_putA (5'-AACAATGCTCTCTCT-GTCGG-3' = SEQ ID No. 19). The resulting fragment, of approx. 2 kb in size, was cloned in an undirected manner into the pCR4-TOPO Invitrogen vector (product number: 45-0030). The resulting plasmid was designated AN 47-196. The sequence of the fragment inserted in plasmid AN 47-196 is depicted under SEQ ID NO 10.

[0215] Primers were now prepared once again in order to amplify a full-length sequence.

[0216] The following primers were used: SOputA (AACAATGCTCTCTCTGTCGG = SEQ ID No. 19) and SO_putE (CACTTTAGAAGGTATCAGAGC = SEQ ID No. 17). A PCR product of approximately 3.2 kb in size was obtained and cloned into the Invitrogen vector pCR2.1 (product number: 45-0030). The resulting plasmid (deposited under DSM 15926) was designated AN 49. The sequence of the fragment inserted in plasmid AN 49 is depicted under SEQ ID NO 11.

[0217] The sequence information in the nucleic acid sequence depicted under SEQ ID No. 13 was obtained by joining the nucleic acid sequences described under SEQ ID NO 9 and SEQ ID NO 10. This nucleic acid sequence is an allelic variant of the nucleic acid sequence described under SEQ ID No 11 encoding a protein (SEQ ID No. 14) which is involved in starch biosynthesis.

Example 3

Producing transgenic potato plants which exhibit a reduced expression of the BEI, SSIII and GBSSI genes and a reduced expression of the gene which is specified under SEQ ID No. 11 or SEQ ID No. 13

[0218] Agrobacteria were used, as described in Rocha-Sosa et al. (EMBO J. 8, (1989), 23-29), to the T-DNA of the plasmid AN 54-196 (see below) into the potato plants having a reduced expression of the BEI, SSIII and GBSSI genes, which plants were described in example 1 and designated, in that example, as asBEI-asSSIII-asGBSSI plants. The plants which were obtained by transformation with plasmid AN 54-196 were designated asBEI-asSSIII-asGBSSI-iBE3 and, in addition to exhibiting reduced expression of the BEI, SSIII and GBSSI genes, also exhibited a reduction in the expression of the gene described under SEQ ID No. 11 or SEQ ID No. 13. Tissue samples were taken from tubers derived from independent transformants, stained with iodine and examined under the microscope. The phosphate content in the C6 position was also determined. The starches from the independent lines whose granules stained brown and which exhibited a phosphate content which was greater than that of the starting lines (see example 1) were used for further analysis of the starch properties.

Information concerning vector AN 54-196

[0219] AN 54-196 is a derivative of plasmid pBinB33-Hyg, into which a constituent sequence of the coding nucleic acid sequence specified under SEQ ID NO 11 or SEQ ID NO 13 was inserted as an inverted repeat (RNAi technology) under the control of the promoter of the *Solanum tuberosum* patatin gene B33 (Rocha-Sosa et al., 1989). For this, a PCR product was first of all amplified from a tuber-specific *Solanum tuberosum* (cv Désirée) cDNA library using the primers B1_Asp (GAT GGG TAC CAG CAC TTC TAC TTG GCA GAG G) and B2_Sal (TCA AGT CGA CCA CAA CCA GTC CAT TTC TGG) resulting in the cleavage sites *Asp*718 and *Sal*I being added. The PCR product (625 bp) which was obtained was cloned, in the antisense orientation with regard to the B33 promoter, by way of these two cleavage sites. A second PCR fragment, which was amplified from a tuber-specific *Solanum tuberosum* (cv Désirée) cDNA library using the primers B3_Sal (GCT TGT CGA CGG GAG AAT TTT GTC CAG AGG = SEQ ID No. 20) and B4_Sal (GAT CGT CGA CAG CAC TTC TAC TTG GCA GAG G = SEQ ID No. 21), and which was identical to 301 bp of the first

fragment, was cloned, by way of the *SalI* cleavage site, downstream of the first fragment but in the sense orientation with regard to the B33 promoter. This arrangement is designated an inverted repeat (RNAi technology).

Information concerning vector pBinB33-Hyg

[0220] The *Eco*RI-*Hind*III fragment comprising the B33 promoter, a part of the polylinker and the ocs terminator, was excised from plasmid pBinB33 and ligated into the vector pBIB-Hyg (Becker, 1990), which had been cut correspondingly.
[0221] The plasmid pBinB33 was obtained by ligating the promoter of the *Solanum tuberosum* patatin gene B33 (Rocha-Sosa et al., 1989), as a *Dra*I fragment (nucleotides -1512 - +14), into the *Sst*I-cut vector pUC19, whose ends had been smoothed using T4 DNA polymerase. This resulted in the plasmid pUC19-B33. The B33 promoter was excised from this plasmid using *Eco*RI and *Sma*I and ligated into vector pBinAR, which had been cut correspondingly. This resulted in the plant expression vector pBinB33.
[0222] The plasmid pBinAR is a derivative of the vector plasmid pBin19 (Bevan, 1984) and was constructed as follows:

A fragment of 529 bp in length, which comprises nucleotides 6909-7437 of the cauliflower mosaic virus 35S RNA promoter (Pietrzak et al., 1986, Nucleic Acids Research 14, 5857-5868), was isolated, as an *Eco*RI/*Kpn*I fragment, from the plasmid pDH51 (Pietrzak et al., 1986) and ligated between the *Eco*RI and *Kpn*I cleavage sites of the pUC18 polylinker. This resulted in the plasmid pUC18-35S.
A fragment of 192 bp in length, which comprises the polyadenylation signal (3' end) of the *octopine synthase* gene (gene 3) of the T-DNA of the Ti-plasmid pTiACH5 (Gielen et al., 1984) (nucleotides 11749-11939), was isolated from the plasmid pAGV40 (Herrera-Estrella et al., 1983) using the restriction endonucleases *Hind*III and *Pvu*II. After *Ssp*I linkers had been added to the *Pvu*II cleavage site, the fragment was ligated between the *Sph*I and *Hind*III cleavage sites of pUC18-35S. This resulted in the plasmid pA7.

[0223] The entire polylinker, containing the 35S promoter and the ocs terminator, was excised from pA7 using *Eco*RI and *Hind*III and ligated into pBin19, which had been cut correspondingly. This resulted in the plant expression vector pBinAR (Höfgen and Willmitzer, 1990).

Example 4

Analyzing the starch of transgenic potato plants which exhibit reduced expression of the BEI, SSIII and GBSSI genes and reduced expression of the gene which is specified under SEQ ID No. 11 or SEQ ID No. 13

[0224] The starches were isolated from the tubers obtained from different independent lines of the asBEI-asSSIII-asGBSSI-iBE3 potato transformants described in example 3. The physicochemical properties of these starches were then analyzed. The results which were obtained on the basis of the starches which were prepared from the plant cells or plants according to the invention are given below either as absolute values or as percentage values based on starch from corresponding wild-type plant cells or wild-type plants (designated WT starch in that which follows) (table 2). In addition, the table contains starch data from "single" or "double" combinations disclosed in WO 00/08184 and WO 01/12782:

Table 2

	% based on WT starch		
	Phosphate in C6	Amylose	Gel strength
asSSIII	197	123	84
cosSSIII	210	-	83
asBEI	170	91	91
asGBSSI	110	<18	-
asBEI-asSSIII	292	89	100
asGBSSI-asBEI	181	<18	21
asBEI-asSSIII-asGBSSI	360	<18	16
asBEI-asSSIII-asGBSSI-iBE3	462	<18	-

[0225] In addition, the absolute values for the amylose content (determined using the methods of Hovenkamp-Hermelink) and for the phosphate content in the C6 position (method description, see "General methods" above) were determined in the case of the starches from the corresponding wild-type plants (Desiree variety), from the starting line (asBEI-asSSIII-asGBSSI) and from the asBEI-asSSIII-asGBSSI-iBE3 potato plants (table 3):

Table 3: Amylose content and phosphate content in the C6 position

Genotype	C6 phosphate [nmol/mg]	Amylose [% of the total starch]
Desiree (wild type)	12.1	24.1
asBEI-asSSIII-asGBSSI	43.5	< 3
asBEI-asSSIII-asGBSSI-iBE3	56.3	< 3

[0226] The side-chain profile of the potato starch was analyzed by determining the content of a particular group of side chains expressed as a percentage of the total content of all the side chains in the GPC chromatogram (table 4) (see General methods "Using gel permeation chromatography to analyze the side-chain distribution of total starch"):

Table 4: Distribution of the side-chain profile of total starch in the lines asBEI-asSIII, asBEI-asSSIII-asGBSSI asBEI-asSSIII-asGBSSI-iBE3 and the corresponding wild type, divided into groups having different degrees of polymerization

Degree of polymerization (dp)	% based on WT starch		
	asBEI-asSSIII	asBEI-asSSIII-asGBSSI	asBEI-asSSIII-asGBSSIiBE3
dp < 12	119.71	156.57	153.27
dp 12-19	103.83	119.00	147.90
dp 20-25	120.36	131.79	142.80
dp 26-31	127.31	142.19	130.42
dp 32-37	122.60	144.16	120.97
dp 38-43	103.14	130.00	110.77
dp 44-49	83.42	114.95	92.40
dp 50-56	72.51	106.85	93.47
dp 57-62	68.85	105.21	90.28
dp 63-123	56.44	91.78	77.70
dp > 123	104.21	3.97	0.96

[0227] The ratio of the total phosphate content to the phosphate content in the C6 position was also determined:

Genotype	Total phosphate content/ C6 phosphate content
Desiree (wild type)	1.88
asBEI-asSSIII-asGBSSI	1.62
asBEI-asSSIII-asGBSSI-iBE3	1.37

[0228] The gel strength of the potato starches according to the invention was analyzed by the method described above (see General Methods, Method d) "Determining the gel strength (texture analyzer)"):

Genotype	Gel strength (g)
Desiree (wild type)	26,0
asBEI-asSSIII-asGBSSI	3,3
asBEI-asSSIII-asGBSSI-iBE3	4,5

[0229] The shear stability of the potato starches according to the invention was analyzed by the method described above (see General Methods, Method k):

Genotype	Shear stability (%)
Desiree (wild type)	56
asBEI-asSSIII-asGBSSI-iBE3	70

[0230] The peak viscosity of the potato starches according to the invention was analyzed by the Rotovisko method described above (see General Methods, Method l):

Genotype	Peak Viscosity (SKT)
Desiree (wild type)	269
asBEI-asSSIII-asGBSSI	330
asBEI-asSSIII-asGBSSI-iBE3	385

[0231] The freeze/thaw stability of the potato starches according to the invention was analyzed by the method described above (see General Methods, Method g) "Determining the freeze/thaw stability"):

Genotype	freeze/thaw stability (%)
Desiree (wild type)	10,5
asBEI-asSSIII-asGBSSI-iBE3	95,0

SEQUENCE LISTING

[0232]

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<302> Cloning and functional analysis of a cDNA encoding a novel 139 kDa

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Val Tyr Gly Cys Ser Asn Asp Gly Glu Arg Phe Gly Phe Phe Cys His
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His Cys His Asp Trp Ser Ser Ala Pro Val Ala Trp Leu Phe Lys Glu
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Asp Arg Ala Arg  Leu Cys Leu Thr Tyr  Asp Glu Pro Leu Ser  His
           1095                1100                1105

ctg ata tat gct  ggt gct gat ttt att  cta gtt cct tca ata  ttt      3569
Leu Ile Tyr Ala  Gly Ala Asp Phe Ile  Leu Val Pro Ser Ile  Phe
           1110                1115                1120

gag cca tgt gga  cta aca caa ctt acc  gct atg aga tat ggt  tca      3614
Glu Pro Cys Gly  Leu Thr Gln Leu Thr  Ala Met Arg Tyr Gly  Ser
           1125                1130                1135

att cca gtc gtg  cgt aaa act gga gga  ctt tat gat act gta  ttt      3659
Ile Pro Val Val  Arg Lys Thr Gly Gly  Leu Tyr Asp Thr Val  Phe
           1140                1145                1150

gat gtt gac cat  gac aaa gag aga gca  caa cag tgt ggt ctt  gaa      3704
Asp Val Asp His  Asp Lys Glu Arg Ala  Gln Gln Cys Gly Leu  Glu
           1155                1160                1165

cca aat gga ttc  agc ttt gat gga gca  gat gct ggc gga gtt  gat      3749
Pro Asn Gly Phe  Ser Phe Asp Gly Ala  Asp Ala Gly Gly Val  Asp
           1170                1175                1180

tat gct ctg aat  aga gct ctc tct gct  tgg tac gat ggt cgg  gat      3794
Tyr Ala Leu Asn  Arg Ala Leu Ser Ala  Trp Tyr Asp Gly Arg  Asp
           1185                1190                1195

tgg ttc aac tct  tta tgc aag cag gtc  atg gaa caa gat tgg  tct      3839
Trp Phe Asn Ser  Leu Cys Lys Gln Val  Met Glu Gln Asp Trp  Ser
           1200                1205                1210

tgg aac cga cct  gct ctt gat tat ttg  gag ctt tac cat gct  gct      3884
Trp Asn Arg Pro  Ala Leu Asp Tyr Leu  Glu Leu Tyr His Ala  Ala
           1215                1220                1225

aga aag tta gaa  tag ttagtttgtg agatgctagc agaaaaattc acgagatctg    3939
Arg Lys Leu Glu
           1230

caatctgtac aggttcagtg tttgcgtctg gacagctttt ttatttccta tatcaaagta   3999
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taaatcaagt ctacactgag atcaatagca gacagtcctc agttcatttc atttttgtg 4059

caacatatga aagagcttag cctctaataa tgtagtcatt gatgattatt tgttttggga 4119

agaaatgaga aatcaaagga tgcaaaatac tctgaaaaaa aaaaaaaa 4167

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<212> PRT
<213> Solanum tuberosum

<400> 2

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Ser Asn Ala Ile Thr His Leu Lys Ile Lys Pro Phe Leu Gly Phe Val
20 25 30

Ser His Gly Thr Thr Ser Leu Ser Val Gln Ser Ser Ser Trp Arg Lys
35 40 45

Asp Gly Met Val Thr Gly Val Ser Phe Pro Phe Cys Ala Asn Leu Ser
50 55 60

Gly Arg Arg Arg Arg Lys Val Ser Thr Thr Arg Ser Gln Gly Ser Ser
65 70 75 80

Pro Lys Gly Phe Val Pro Arg Lys Pro Ser Gly Met Ser Thr Gln Arg
85 90 95

Lys Val Gln Lys Ser Asn Gly Asp Lys Glu Ser Gln Ser Thr Ser Thr
100 105 110

Ser Lys Glu Ser Glu Ile Ser Asn Gln Lys Thr Val Glu Ala Arg Val
115 120 125

Glu Thr Ser Asp Asp Asp Thr Lys Val Val Val Arg Asp His Lys Phe
130 135 140

Leu Glu Asp Glu Asp Glu Ile Asn Gly Ser Thr Lys Ser Ile Ser Met
145 150 155 160

Ser Pro Val Arg Val Ser Ser Gln Phe Val Glu Ser Glu Glu Thr Gly
165 170 175

Gly Asp Asp Lys Asp Ala Val Lys Leu Asn Lys Ser Lys Arg Ser Glu
180 185 190

Glu Ser Asp Phe Leu Ile Asp Ser Val Ile Arg Glu Gln Ser Gly Ser
195 200 205

Gln Gly Glu Thr Asn Ala Ser Ser Lys Gly Ser His Ala Val Gly Thr
210 215 220

Lys Leu Tyr Glu Ile Leu Gln Val Asp Val Glu Pro Gln Gln Leu Lys
225 230 235 240

Glu Asn Asn Ala Gly Asn Val Glu Tyr Lys Gly Pro Val Ala Ser Lys
245 250 255

Leu Leu Glu Ile Thr Lys Ala Ser Asp Val Glu His Thr Glu Ser Asn
260 265 270

Glu Ile Asp Asp Leu Asp Thr Asn Ser Phe Phe Lys Ser Asp Leu Ile
275 280 285

Glu Glu Asp Glu Pro Leu Ala Ala Gly Thr Val Glu Thr Gly Asp Ser
290 295 300

Ser Leu Asn Leu Arg Leu Glu Met Glu Ala Asn Leu Arg Arg Gln Ala
305 310 315 320

Ile Glu Arg Leu Ala Glu Glu Asn Leu Leu Gln Gly Ile Arg Leu Phe
325 330 335

Cys Phe Pro Glu Val Val Lys Pro Asp Glu Asp Val Glu Ile Phe Leu
340 345 350

Asn Arg Gly Leu Ser Thr Leu Lys Asn Glu Ser Asp Val Leu Ile Met
355 360 365

Gly Ala Phe Asn Glu Trp Arg Tyr Arg Ser Phe Thr Thr Arg Leu Thr
370 375 380

Glu Thr His Leu Asn Gly Asp Trp Trp Ser Cys Lys Ile His Val Pro
385 390 395 400

Lys Glu Ala Tyr Arg Ala Asp Phe Val Phe Phe Asn Gly Gln Asp Val
405 410 415

Tyr Asp Asn Asn Asp Gly Asn Asp Phe Ser Ile Thr Val Lys Gly Gly
420 425 430

Met Gln Ile Ile Asp Phe Glu Asn Phe Leu Leu Glu Glu Lys Trp Arg
435 440 445

Glu Gln Glu Lys Leu Ala Lys Glu Gln Ala Glu Arg Glu Arg Leu Ala
450 455 460

Glu Glu Gln Arg Arg Ile Glu Ala Glu Lys Ala Glu Ile Glu Ala Asp
465 470 475 480

Arg Ala Gln Ala Lys Glu Glu Ala Ala Lys Lys Lys Lys Val Leu Arg
485 490 495

Glu Leu Met Val Lys Ala Thr Lys Thr Arg Asp Ile Thr Trp Tyr Ile
500 505 510

Glu Pro Ser Glu Phe Lys Cys Glu Asp Lys Val Arg Leu Tyr Tyr Asn
515 520 525

Lys Ser Ser Gly Pro Leu Ser His Ala Lys Asp Leu Trp Ile His Gly
530 535 540

Gly Tyr Asn Asn Trp Lys Asp Gly Leu Ser Ile Val Lys Lys Leu Val
545 550 555 560

Lys Ser Glu Arg Ile Asp Gly Asp Trp Trp Tyr Thr Glu Val Val Ile
565 570 575

Pro Asp Gln Ala Leu Phe Leu Asp Trp Val Phe Ala Asp Gly Pro Pro
580 585 590

Lys His Ala Ile Ala Tyr Asp Asn Asn His Arg Gln Asp Phe His Ala
595 600 605

Ile Val Pro Asn His Ile Pro Glu Glu Leu Tyr Trp Val Glu Glu Glu
610 615 620

His Gln Ile Phe Lys Thr Leu Gln Glu Glu Arg Arg Leu Arg Glu Ala
625 630 635 640

Ala Met Arg Ala Lys Val Glu Lys Thr Ala Leu Leu Lys Thr Glu Thr
645 650 655

Lys Glu Arg Thr Met Lys Ser Phe Leu Leu Ser Gln Lys His Val Val
660 665 670

Tyr Thr Glu Pro Leu Asp Ile Gln Ala Gly Ser Ser Val Thr Val Tyr
675 680 685

Tyr Asn Pro Ala Asn Thr Val Leu Asn Gly Lys Pro Glu Ile Trp Phe
690 695 700

Arg Cys Ser Phe Asn Arg Trp Thr His Arg Leu Gly Pro Leu Pro Pro
705 710 715 720

Gln Lys Met Ser Pro Ala Glu Asn Gly Thr His Val Arg Ala Thr Val
725 730 735

Lys Val Pro Leu Asp Ala Tyr Met Met Asp Phe Val Phe Ser Glu Arg
740 745 750

Glu Asp Gly Gly Ile Phe Asp Asn Lys Ser Gly Met Asp Tyr His Ile
755 760 765

Pro Val Phe Gly Gly Val Ala Lys Glu Pro Pro Met His Ile Val His
770 775 780

Ile Ala Val Glu Met Ala Pro Ile Ala Lys Val Gly Gly Leu Gly Asp
785 790 795 800

Val Val Thr Ser Leu Ser Arg Ala Val Gln Asp Leu Asn His Asn Val
805 810 815

Asp Ile Ile Leu Pro Lys Tyr Asp Cys Leu Lys Met Asn Asn Val Lys
820 825 830

Asp Phe Arg Phe His Lys Asn Tyr Phe Trp Gly Gly Thr Glu Ile Lys
835 840 845

Val Trp Phe Gly Lys Val Glu Gly Leu Ser Val Tyr Phe Leu Glu Pro
850 855 860

Gln Asn Gly Leu Phe Ser Lys Gly Cys Val Tyr Gly Cys Ser Asn Asp
865 870 875 880

Gly Glu Arg Phe Gly Phe Phe Cys His Ala Ala Leu Glu Phe Leu Leu
885 890 895

Gln Gly Gly Phe Ser Pro Asp Ile Ile His Cys His Asp Trp Ser Ser
900 905 910

Ala Pro Val Ala Trp Leu Phe Lys Glu Gln Tyr Thr His Tyr Gly Leu
915 920 925

Ser Lys Ser Arg Ile Val Phe Thr Ile His Asn Leu Glu Phe Gly Ala
930 935 940

Asp Leu Ile Gly Arg Ala Met Thr Asn Ala Asp Lys Ala Thr Thr Val
945 950 955 960

Ser Pro Thr Tyr Ser Gln Glu Val Ser Gly Asn Pro Val Ile Ala Pro
965 970 975

His Leu His Lys Phe His Gly Ile Val Asn Gly Ile Asp Pro Asp Ile
980 985 990

Trp Asp Pro Leu Asn Asp Lys Phe Ile Pro Ile Pro Tyr Thr Ser Glu
995 1000 1005

Asn Val Val Glu Gly Lys Thr Ala Ala Lys Glu Ala Leu Gln Arg
1010 1015 1020

Lys Leu Gly Leu Lys Gln Ala Asp Leu Pro Leu Val Gly Ile Ile
1025 1030 1035

Thr Arg Leu Thr His Gln Lys Gly Ile His Leu Ile Lys His Ala
1040 1045 1050

Ile Trp Arg Thr Leu Glu Arg Asn Gly Gln Val Val Leu Leu Gly
1055 1060 1065

Ser Ala Pro Asp Pro Arg Val Gln Asn Asp Phe Val Asn Leu Ala
1070 1075 1080

Asn Gln Leu His Ser Lys Tyr Asn Asp Arg Ala Arg Leu Cys Leu
1085 1090 1095

Thr Tyr Asp Glu Pro Leu Ser His Leu Ile Tyr Ala Gly Ala Asp
1100 1105 1110

Phe Ile Leu Val Pro Ser Ile Phe Glu Pro Cys Gly Leu Thr Gln
1115 1120 1125

Leu Thr Ala Met Arg Tyr Gly Ser Ile Pro Val Val Arg Lys Thr
1130 1135 1140

Gly Gly Leu Tyr Asp Thr Val Phe Asp Val Asp His Asp Lys Glu
1145 1150 1155

Arg Ala Gln Gln Cys Gly Leu Glu Pro Asn Gly Phe Ser Phe Asp
1160 1165 1170

Gly Ala Asp Ala Gly Gly Val Asp Tyr Ala Leu Asn Arg Ala Leu
1175 1180 1185

Ser Ala Trp Tyr Asp Gly Arg Asp Trp Phe Asn Ser Leu Cys Lys
1190 1195 1200

Gln Val Met Glu Gln Asp Trp Ser Trp Asn Arg Pro Ala Leu Asp
1205 1210 1215

Tyr Leu Glu Leu Tyr His Ala Ala Arg Lys Leu Glu
1220 1225 1230

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<212> PRT
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<400> 3

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Val Phe Phe Asn Gly Gln Asp Val Tyr Asp Asn Asn Asp Gly Asn Asp
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Phe Ser Ile Thr Val Lys Gly Gly Met Gln Ile Ile Asp
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<212> DNA
<213> Solanum tuberosum

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ccttatctag atcacttcag acacagaatg aagagatatg tggatcagaa aatgctcatt    180
gaaaaatatg agggacccct tgaggaattt gctcaaggtt atttaaaatt tggattcaac    240
agggaagatg gttgcatagt ctatcgtgaa tgggctcctg ctgctcagga agcagaagtt    300
attggcgatt tcaatggtag gaacggttct aaccacatga tggagaagga ccagtttggt    360
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atggccataa tggaacattc ttactatgga tcatttggat atcatgttac aaacttttt     780
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cttcgtttcc ttctttccaa cttgaggtgg tggctagaag agtataactt tgacggattt   1080
cgatttgatg gaataacttc tatgctgtat gttcatcatg gaatcaatat gggatttaca   1140
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tctggtatgc cgggccttag ccggcctgtt tctgagggag gaattggttt tgattaccgc 1320

ctggcaatgg caatcccaga taagtggata gattatttaa agaataagaa tgatgaagat 1380

tggtccatga aggaagtaac atcgagtttg acaaatagga gatatacaga gaagtgtata 1440

gcatatgcgg agagccatga tcagtctatt gtcggtgaca agaccattgc atttctccta 1500

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<212> PRT
<213> Solanum tuberosum

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<309> 26.07.1993

<400> 5

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Thr Met Ala Pro Leu Glu Glu Asp Val Asn Thr Glu Asn Ile Gly Leu
20 25 30

Leu Asn Leu Asp Pro Thr Leu Glu Pro Tyr Leu Asp His Phe Arg His
35 40 45

Arg Met Lys Arg Tyr Val Asp Gln Lys Met Leu Ile Glu Lys Tyr Glu
50 55 60

Gly Pro Leu Glu Glu Phe Ala Gln Gly Tyr Leu Lys Phe Gly Phe Asn
65 70 75 80

Arg Glu Asp Gly Cys Ile Val Tyr Arg Glu Trp Ala Pro Ala Ala Gln
85 90 95

Glu Ala Glu Val Ile Gly Asp Phe Asn Gly Arg Asn Gly Ser Asn His
100 105 110

Met Met Glu Lys Asp Gln Phe Gly Val Trp Ser Ile Arg Ile Pro Asp
115 120 125

Val Asp Ser Lys Pro Val Ile Pro His Asn Ser Arg Val Lys Phe Arg
130 135 140

Phe Lys His Gly Asn Gly Val Trp Val Asp Arg Ile Pro Ala Trp Ile
145 150 155 160

Lys Tyr Ala Thr Ala Asp Ala Thr Lys Phe Ala Ala Pro Tyr Asp Gly
165 170 175

Val Tyr Trp Asp Pro Pro Pro Ser Glu Arg Tyr His Phe Lys Tyr Pro
180 185 190

Arg Pro Pro Lys Pro Arg Ala Pro Arg Ile Tyr Glu Ala His Val Gly
195 200 205

Met Ser Ser Ser Glu Pro Arg Val Asn Ser Tyr Arg Glu Phe Ala Asp
210 215 220

Asp Val Leu Pro Arg Ile Lys Ala Asn Asn Tyr Asn Thr Val Gln Leu
225 230 235 240

Met Ala Ile Met Glu His Ser Tyr Tyr Gly Ser Phe Gly Tyr His Val
245 250 255

Thr Asn Phe Phe Ala Val Ser Asn Arg Tyr Gly Asn Pro Glu Asp Leu
260 265 270

Lys Tyr Leu Ile Asp Lys Ala His Ser Leu Gly Leu Gln Val Leu Val
275 280 285

Asp Val Val His Ser His Ala Ser Asn Asn Val Thr Asp Gly Leu Asn
290 295 300

Gly Phe Asp Ile Gly Gln Gly Ser Gln Glu Ser Tyr Phe His Ala Gly
305 310 315 320

Glu Arg Gly Tyr His Lys Leu Trp Asp Ser Arg Leu Phe Asn Tyr Ala
325 330 335

Asn Trp Glu Val Leu Arg Phe Leu Leu Ser Asn Leu Arg Trp Trp Leu
340 345 350

Glu Glu Tyr Asn Phe Asp Gly Phe Arg Phe Asp Gly Ile Thr Ser Met
355 360 365

Leu Tyr Val His His Gly Ile Asn Met Gly Phe Thr Gly Asn Tyr Asn
370 375 380

Glu Tyr Phe Ser Glu Ala Thr Asp Val Asp Ala Val Val Tyr Leu Met
385 390 395 400

Leu Ala Asn Asn Leu Ile His Lys Ile Phe Pro Asp Ala Thr Val Ile
405 410 415

Ala Glu Asp Val Ser Gly Met Pro Gly Leu Ser Arg Pro Val Ser Glu
420 425 430

Gly Gly Ile Gly Phe Asp Tyr Arg Leu Ala Met Ala Ile Pro Asp Lys
435 440 445

Trp Ile Asp Tyr Leu Lys Asn Lys Asn Asp Glu Asp Trp Ser Met Lys
450 455 460

Glu Val Thr Ser Ser Leu Thr Asn Arg Arg Tyr Thr Glu Lys Cys Ile
465 470 475 480

Ala Tyr Ala Glu Ser His Asp Gln Ser Ile Val Gly Asp Lys Thr Ile
485 490 495

Ala Phe Leu Leu Met Asn Lys Glu Met Tyr Ser Gly Met Ser Cys Leu
500 505 510

Thr Asp Ala Ser Pro Val Val Asp Ala Gly Ile Ala Leu Asp Lys Met
515 520 525

Ile His Phe Phe His Asn Gly Leu Gly Arg Arg Gly Val Pro Gln Phe
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His Gly
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<212> DNA
<213> Solanum tuberosum

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<211> 607
<212> PRT
<213> Solanum tuberosum

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35 40 45

Gln Ser Arg Thr Asn Thr Lys Val Thr Pro Lys Met Ala Ser Arg Thr
50 55 60

Glu Thr Lys Arg Pro Gly Cys Ser Ala Thr Ile Val Cys Gly Lys Gly
65 70 75 80

Met Asn Leu Ile Phe Val Gly Thr Glu Val Gly Pro Trp Ser Lys Thr
85 90 95

Gly Gly Leu Gly Asp Val Leu Gly Gly Leu Pro Pro Ala Leu Ala Ala
100 105 110

Arg Gly His Arg Val Met Thr Ile Ser Pro Arg Tyr Asp Gln Tyr Lys
115 120 125

Asp Ala Trp Asp Thr Ser Val Ala Val Glu Val Lys Val Gly Asp Ser
130 135 140

Ile Glu Ile Val Arg Phe Phe His Cys Tyr Lys Arg Gly Val Asp Arg
145 150 155 160

Val Phe Val Asp His Pro Met Phe Leu Glu Lys Val Trp Gly Lys Thr
165 170 175

Gly Ser Lys Ile Tyr Gly Pro Lys Ala Gly Leu Asp Tyr Leu Asp Asn
180 185 190

Glu Leu Arg Phe Ser Leu Leu Cys Gln Ala Ala Leu Glu Ala Pro Lys
195 200 205

Val Leu Asn Leu Asn Ser Ser Asn Tyr Phe Ser Gly Pro Tyr Gly Glu
210 215 220

Asp Val Leu Phe Ile Ala Asn Asp Trp His Thr Ala Leu Ile Pro Cys
225 230 235 240

Tyr Leu Lys Ser Met Tyr Gln Ser Arg Gly Ile Tyr Leu Asn Ala Lys
245 250 255

Val Ala Phe Cys Ile His Asn Ile Ala Tyr Gln Gly Arg Phe Ser Phe
260 265 270

Ser Asp Phe Pro Leu Leu Asn Leu Pro Asp Glu Phe Arg Gly Ser Phe
275 280 285

Asp Phe Ile Asp Gly Tyr Glu Lys Pro Val Lys Gly Arg Lys Ile Asn
290 295 300

Trp Met Lys Ala Gly Ile Leu Glu Ser His Arg Val Val Thr Val Ser
305 310 315 320

Pro Tyr Tyr Ala Gln Glu Leu Val Ser Ala Val Asp Lys Gly Val Glu
325 330 335

Leu Asp Ser Val Leu Arg Lys Thr Cys Ile Thr Gly Ile Val Asn Gly
340 345 350

Met Asp Thr Gln Glu Trp Asn Pro Ala Thr Asp Lys Tyr Thr Asp Val
355 360 365

Lys Tyr Asp Ile Thr Thr Val Met Asp Ala Lys Pro Leu Leu Lys Glu
370 375 380

Ala Leu Gln Ala Ala Val Gly Leu Pro Val Asp Lys Lys Ile Pro Leu
385 390 395 400

Ile Gly Phe Ile Gly Arg Leu Glu Glu Gln Lys Gly Ser Asp Ile Leu
405 410 415

Val Ala Ala Ile His Lys Phe Ile Gly Leu Asp Val Gln Ile Val Val
420 425 430

Leu Gly Thr Gly Lys Lys Glu Phe Glu Gln Glu Ile Glu Gln Leu Glu
435 440 445

Val Leu Tyr Pro Asn Lys Ala Lys Gly Val Ala Lys Phe Asn Val Pro
450 455 460

Leu Ala His Met Ile Thr Ala Gly Ala Asp Phe Met Leu Val Pro Ser
465 470 475 480

Arg Phe Glu Pro Cys Gly Leu Ile Gln Leu His Ala Met Arg Tyr Gly
485 490 495

Thr Val Pro Ile Cys Ala Ser Thr Gly Gly Leu Val Asp Thr Val Lys
500 505 510

Glu Gly Tyr Thr Gly Phe His Met Gly Ala Phe Asn Val Glu Cys Asp
515 520 525

Val Val Asp Pro Ala Asp Val Leu Lys Ile Val Thr Thr Val Ala Arg
530 535 540

Ala Leu Ala Val Tyr Gly Thr Leu Ala Phe Ala Glu Met Ile Lys Asn
545 550 555 560

Cys Met Ser Glu Glu Leu Ser Trp Lys Glu Pro Ala Lys Lys Trp Glu
565 570 575

Thr Leu Leu Leu Gly Leu Gly Ala Ser Gly Ser Glu Pro Gly Val Glu
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Gly Glu Glu Ile Ala Pro Leu Ala Lys Glu Asn Val Ala Thr Pro
595 600 605

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<210> 9
<211> 1004
<212> DNA
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<400> 9

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<211> 2096
<212> DNA
<213> Solanum tuberosum

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gcactttctt ctttcaaatc tgaactggtg ggtggaggag tatcatgtcg atggcttcca 1680

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catggatgaa tactgtaacc aatatgttga caaggaggcc ttattgtacc tcatattagc 1800

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tctttctgcc tcagagatgt ggcttgcatt acttgaaaat actcctgatc atgaatggtg 1980

catgagtaag attgttagca cattagtggg cgatagacaa aatactgata aaatgctttt 2040

gtatgcagaa aatcacaacc agtccatttc tggaggtcgt tccttcgcag aaatac 2096

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Met Leu Ser Leu Ser Asp
1 5

tca att cga att tct tca cca ttg agc gat tct cgt ctt agt ttt cta 164
Ser Ile Arg Ile Ser Ser Pro Leu Ser Asp Ser Arg Leu Ser Phe Leu
10 15 20

tct caa acc gga agc aga acc agt cgc cag ctt aaa ttt gtt cgc agc 212
Ser Gln Thr Gly Ser Arg Thr Ser Arg Gln Leu Lys Phe Val Arg Ser
25 30 35

cgc cgg gct cga gtt tcg agg tgt aga tgc tca gca acg gag caa ccg 260
Arg Arg Ala Arg Val Ser Arg Cys Arg Cys Ser Ala Thr Glu Gln Pro
40 45 50

cca ccg caa cga cgg aag caa cga ccg gag aag tac aaa cag tcg gag 308
Pro Pro Gln Arg Arg Lys Gln Arg Pro Glu Lys Tyr Lys Gln Ser Glu
55 60 65 70

gaa gag aaa gga atc gat cct gtt gga ttt ctc agc aaa tac ggc att 356
Glu Glu Lys Gly Ile Asp Pro Val Gly Phe Leu Ser Lys Tyr Gly Ile
75 80 85

act cat aaa gcg ttt gct caa ttt ctt cgt gaa aga tat aaa tca ttg 404

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Thr His Lys Ala Phe Ala Gln Phe Leu Arg Glu Arg Tyr Lys Ser Leu
                90                  95                 100

aag gac ttg aag gat gaa ata ttg act cgt cat ttc agt ctc aag gag      452
Lys Asp Leu Lys Asp Glu Ile Leu Thr Arg His Phe Ser Leu Lys Glu
        105                 110                 115

atg tct act ggg tat gaa tta atg ggt atg cat cgc aac ata caa cat      500
Met Ser Thr Gly Tyr Glu Leu Met Gly Met His Arg Asn Ile Gln His
    120                 125                 130

cga gtg gat ttc ttg gaa tgg gct cca ggt gct cgc tac tgt gct ctg      548
Arg Val Asp Phe Leu Glu Trp Ala Pro Gly Ala Arg Tyr Cys Ala Leu
135                 140                 145                 150

att ggt gac ttc aat ggg tgg tca aca act ggt aac tgt gcc aga gag      596
Ile Gly Asp Phe Asn Gly Trp Ser Thr Thr Gly Asn Cys Ala Arg Glu
                155                 160                 165

ggt cat ttt ggt cat gac gat tat ggg tat tgg ttt att att ctt gaa      644
Gly His Phe Gly His Asp Asp Tyr Gly Tyr Trp Phe Ile Ile Leu Glu
            170                 175                 180

gat aaa tta cgt gaa gga gaa gaa cct gat aaa ttg tat ttt caa cag      692
Asp Lys Leu Arg Glu Gly Glu Glu Pro Asp Lys Leu Tyr Phe Gln Gln
        185                 190                 195

tac aat tat gcg gag gac tat gat aaa ggt gac acg ggt att acc gtc      740
Tyr Asn Tyr Ala Glu Asp Tyr Asp Lys Gly Asp Thr Gly Ile Thr Val
    200                 205                 210

gag gaa atc ttt aaa aaa gca aat gat gag tat tgg gaa cct gga gaa      788
Glu Glu Ile Phe Lys Lys Ala Asn Asp Glu Tyr Trp Glu Pro Gly Glu
215                 220                 225                 230

gat cgc ttc att aaa tca cgt tat gag gtg gca gca aag tta tat gag      836
Asp Arg Phe Ile Lys Ser Arg Tyr Glu Val Ala Ala Lys Leu Tyr Glu
                235                 240                 245

gaa atg ttc gga cca aat gga cct caa aca gaa gag gaa cta gaa gca      884
Glu Met Phe Gly Pro Asn Gly Pro Gln Thr Glu Glu Glu Leu Glu Ala
            250                 255                 260

atg cct gat gca gct aca cga tac aaa act tgg aaa gag caa caa aaa      932
Met Pro Asp Ala Ala Thr Arg Tyr Lys Thr Trp Lys Glu Gln Gln Lys
        265                 270                 275

aag gat ccg gca agc aat ttg cca tcg tat gat gtg gta gat agt gga      980
Lys Asp Pro Ala Ser Asn Leu Pro Ser Tyr Asp Val Val Asp Ser Gly
    280                 285                 290

aaa gaa tat gat att tac aat att ata ggt gat cct gaa tcg ttt aag     1028
Lys Glu Tyr Asp Ile Tyr Asn Ile Ile Gly Asp Pro Glu Ser Phe Lys
295                 300                 305                 310

aaa ttt cgt atg aaa cag cct cct att gct tac tgg tta gaa act aaa     1076
Lys Phe Arg Met Lys Gln Pro Pro Ile Ala Tyr Trp Leu Glu Thr Lys
                315                 320                 325

aag gga agg aaa ggc tgg tta cag aaa tat atg cct gct tta cct cat     1124
Lys Gly Arg Lys Gly Trp Leu Gln Lys Tyr Met Pro Ala Leu Pro His
            330                 335                 340

gga agc aaa cac agg gtg tat ttt aac aca cca aat ggg cct ctt gaa     1172
Gly Ser Lys His Arg Val Tyr Phe Asn Thr Pro Asn Gly Pro Leu Glu
        345                 350                 355

cga gtt cct gcg tgg gcc aat ttt gtc att cca gat gca gac ggg atg     1220
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Arg Val Pro Ala Trp Ala Asn Phe Val Ile Pro Asp Ala Asp Gly Met
    360                 365                 370

gca tta gca gtc cat tgg gaa cca cct cct gaa tat gct tat aaa tgg   1268
Ala Leu Ala Val His Trp Glu Pro Pro Pro Glu Tyr Ala Tyr Lys Trp
375                 380                 385                 390

aaa cac aag cta cca gtc aag cct aag tcc ttg cgc ata tat gaa tgt   1316
Lys His Lys Leu Pro Val Lys Pro Lys Ser Leu Arg Ile Tyr Glu Cys
                395                 400                 405

cat gtt ggc atc tct ggc cag gaa cca aaa gtt tca tct ttc aat gat   1364
His Val Gly Ile Ser Gly Gln Glu Pro Lys Val Ser Ser Phe Asn Asp
            410                 415                 420

ttt att agc aag gtc ctt ccg cat gta aaa gaa gct gga tac aat gca   1412
Phe Ile Ser Lys Val Leu Pro His Val Lys Glu Ala Gly Tyr Asn Ala
        425                 430                 435

acg caa att att gga gtt gtt gag cac aag gat tat ttc act gtt gga   1460
Thr Gln Ile Ile Gly Val Val Glu His Lys Asp Tyr Phe Thr Val Gly
    440                 445                 450

tat aga gtg acc aat ttt tat gct gtt agt agc cgt tat ggc aca ccg   1508
Tyr Arg Val Thr Asn Phe Tyr Ala Val Ser Ser Arg Tyr Gly Thr Pro
455                 460                 465                 470

gat gac ttc aag cgc ttg gtt gat gaa gca cat ggg ctt gga ctg ctt   1556
Asp Asp Phe Lys Arg Leu Val Asp Glu Ala His Gly Leu Gly Leu Leu
                475                 480                 485

gtc ttt ttg gag att gtg cac tcc tat gca gca gca gat gaa atg gtt   1604
Val Phe Leu Glu Ile Val His Ser Tyr Ala Ala Ala Asp Glu Met Val
            490                 495                 500

ggg tta tct ctt ttt gat gga gca aat gat tgc tat ttc cac act ggt   1652
Gly Leu Ser Leu Phe Asp Gly Ala Asn Asp Cys Tyr Phe His Thr Gly
        505                 510                 515

aaa cgt gga cac cac aaa ttc tgg ggc aca cgg atg ttc aaa tat gga   1700
Lys Arg Gly His His Lys Phe Trp Gly Thr Arg Met Phe Lys Tyr Gly
    520                 525                 530

gat cct gat gtt ctg cac ttt ctt ctt tca aat ctg aac tgg tgg gtg   1748
Asp Pro Asp Val Leu His Phe Leu Leu Ser Asn Leu Asn Trp Trp Val
535                 540                 545                 550

gag gag tat cat gtc gat ggc ttc cat ttt cat tcg ctc tcg tcc atg   1796
Glu Glu Tyr His Val Asp Gly Phe His Phe His Ser Leu Ser Ser Met
                555                 560                 565

ttg tat acg cat aat gga ttt gct tca ttt act ggt gac atg gat gaa   1844
Leu Tyr Thr His Asn Gly Phe Ala Ser Phe Thr Gly Asp Met Asp Glu
            570                 575                 580

tac tgt aac caa tat gtt gac aag gag gcc tta ttg tac ctc ata tta   1892
Tyr Cys Asn Gln Tyr Val Asp Lys Glu Ala Leu Leu Tyr Leu Ile Leu
        585                 590                 595

gca aat gaa gta tta cat gct ctt cat cct aat gtg atc acg att gct   1940
Ala Asn Glu Val Leu His Ala Leu His Pro Asn Val Ile Thr Ile Ala
    600                 605                 610

gtg gat gca act ctg tat cct gga ctc tgc gat cca aca tct caa ggt   1988
Val Asp Ala Thr Leu Tyr Pro Gly Leu Cys Asp Pro Thr Ser Gln Gly
615                 620                 625                 630

gga ctg ggc ttt gat tat ttt gcc aat ctt tct gcc tca gag atg tgg   2036
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Gly Leu Gly Phe Asp Tyr Phe Ala Asn Leu Ser Ala Ser Glu Met Trp
                635                 640                 645

ctt gca tta ctt gaa aat act cct gat cat gaa tgg tgc atg agt aag      2084
Leu Ala Leu Leu Glu Asn Thr Pro Asp His Glu Trp Cys Met Ser Lys
            650                 655                 660

att gtt agc aca tta gtg ggc gat aga caa aat act gat aaa atg ctt      2132
Ile Val Ser Thr Leu Val Gly Asp Arg Gln Asn Thr Asp Lys Met Leu
        665                 670                 675

ttg tat gca gaa aat cac aac cag tcc att tct gga ggt cgt tcc ttc      2180
Leu Tyr Ala Glu Asn His Asn Gln Ser Ile Ser Gly Gly Arg Ser Phe
    680                 685                 690

gca gaa ata ctg att ggt aac tcc ttg ggg aaa tct tcc ata tca caa      2228
Ala Glu Ile Leu Ile Gly Asn Ser Leu Gly Lys Ser Ser Ile Ser Gln
695                 700                 705                 710

gag tca tta ctt aga ggc tgc tcg tta cac aag atg atc aga tta att      2276
Glu Ser Leu Leu Arg Gly Cys Ser Leu His Lys Met Ile Arg Leu Ile
                715                 720                 725

aca tct aca att ggt ggt cat gca tac ctc aac ttc atg ggc aat gaa      2324
Thr Ser Thr Ile Gly Gly His Ala Tyr Leu Asn Phe Met Gly Asn Glu
            730                 735                 740

ttt ggt cac cca aag aga gta gag ttt cca atg tca agc aac aat ttc      2372
Phe Gly His Pro Lys Arg Val Glu Phe Pro Met Ser Ser Asn Asn Phe
        745                 750                 755

tcc ttt tca ctg gct aac cgt cgc tgg gat cta ttg gaa gat gtt gta      2420
Ser Phe Ser Leu Ala Asn Arg Arg Trp Asp Leu Leu Glu Asp Val Val
    760                 765                 770

cat tat caa tta ttc tca ttt gat aag gat atg atg gac ttg gat aaa      2468
His Tyr Gln Leu Phe Ser Phe Asp Lys Asp Met Met Asp Leu Asp Lys
775                 780                 785                 790

aat ggg aga att ttg tcc aga ggt ctt gcc aac att cac cat gtc aat      2516
Asn Gly Arg Ile Leu Ser Arg Gly Leu Ala Asn Ile His His Val Asn
                795                 800                 805

gat act acc atg gtg att tct tac ttg aga ggt ccc aat ctc ttt gtg      2564
Asp Thr Thr Met Val Ile Ser Tyr Leu Arg Gly Pro Asn Leu Phe Val
            810                 815                 820

ttc aac ttt cat cct gtc aat tca tat gaa aga tac att ata ggt gtg      2612
Phe Asn Phe His Pro Val Asn Ser Tyr Glu Arg Tyr Ile Ile Gly Val
        825                 830                 835

gaa gaa gct gga gag tat caa gtc aca tta aat aca gat gaa aac aag      2660
Glu Glu Ala Gly Glu Tyr Gln Val Thr Leu Asn Thr Asp Glu Asn Lys
    840                 845                 850

tat ggt ggt aga gga cta ctt ggc cat gat cag aat act caa aga acc      2708
Tyr Gly Gly Arg Gly Leu Leu Gly His Asp Gln Asn Thr Gln Arg Thr
855                 860                 865                 870

att agt aga aga gct gat gga atg aga ttt tgc ttg gaa gta cct ctg      2756
Ile Ser Arg Arg Ala Asp Gly Met Arg Phe Cys Leu Glu Val Pro Leu
                875                 880                 885

cca agt aga agt gct cag gtc tac aag ttg acc cga att cta aga gca      2804
Pro Ser Arg Ser Ala Gln Val Tyr Lys Leu Thr Arg Ile Leu Arg Ala
            890                 895                 900

tgatcactct agcaatcaaa gtgcctcata tgatcacaca aaagggaagg ttctacattg    2864
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cccttatact gaccaatatt gtggcctttc cgaggtgagt ttctgtgatt cttgagcaca 2924

ggctgttggc tagtcagtta tcatgaactt ttgccttcag catctggata agcgcttctc 2984

ctgtgcaatg agggcatgga cgaaattttt ttggttcgtc atgggagtca aaagcatctg 3044

ccagattaag atttcacagg cctcgagtaa aaccatcact tacttaggat acacaaacac 3104

atcaacgggg tgcaggctct gataccttct aaagtgaagc cgaattccag cacactggcg 3164

gccgttacta gtggatccga gctcggtacc aagcttggcg 3204

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<212> PRT
<213> Solanum tuberosum

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            20                  25                  30

Leu Lys Phe Val Arg Ser Arg Arg Ala Arg Val Ser Arg Cys Arg Cys
        35                  40                  45

Ser Ala Thr Glu Gln Pro Pro Pro Gln Arg Arg Lys Gln Arg Pro Glu
    50                  55                  60

Lys Tyr Lys Gln Ser Glu Glu Glu Lys Gly Ile Asp Pro Val Gly Phe
65                  70                  75                  80

Leu Ser Lys Tyr Gly Ile Thr His Lys Ala Phe Ala Gln Phe Leu Arg
                85                  90                  95

Glu Arg Tyr Lys Ser Leu Lys Asp Leu Lys Asp Glu Ile Leu Thr Arg
            100                 105                 110

His Phe Ser Leu Lys Glu Met Ser Thr Gly Tyr Glu Leu Met Gly Met
        115                 120                 125

His Arg Asn Ile Gln His Arg Val Asp Phe Leu Glu Trp Ala Pro Gly
    130                 135                 140

Ala Arg Tyr Cys Ala Leu Ile Gly Asp Phe Asn Gly Trp Ser Thr Thr
145                 150                 155                 160

Gly Asn Cys Ala Arg Glu Gly His Phe Gly His Asp Asp Tyr Gly Tyr
                165                 170                 175
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Trp Phe Ile Ile Leu Glu Asp Lys Leu Arg Glu Gly Glu Glu Pro Asp
            180                 185                 190

Lys Leu Tyr Phe Gln Gln Tyr Asn Tyr Ala Glu Asp Tyr Asp Lys Gly
        195                 200                 205

Asp Thr Gly Ile Thr Val Glu Glu Ile Phe Lys Lys Ala Asn Asp Glu
    210                 215                 220

Tyr Trp Glu Pro Gly Glu Asp Arg Phe Ile Lys Ser Arg Tyr Glu Val
225                 230                 235                 240

Ala Ala Lys Leu Tyr Glu Glu Met Phe Gly Pro Asn Gly Pro Gln Thr
                245                 250                 255

Glu Glu Glu Leu Glu Ala Met Pro Asp Ala Ala Thr Arg Tyr Lys Thr
            260                 265                 270

Trp Lys Glu Gln Gln Lys Lys Asp Pro Ala Ser Asn Leu Pro Ser Tyr
        275                 280                 285

Asp Val Val Asp Ser Gly Lys Glu Tyr Asp Ile Tyr Asn Ile Ile Gly
    290                 295                 300

Asp Pro Glu Ser Phe Lys Lys Phe Arg Met Lys Gln Pro Pro Ile Ala
305                 310                 315                 320

Tyr Trp Leu Glu Thr Lys Lys Gly Arg Lys Gly Trp Leu Gln Lys Tyr
                325                 330                 335

Met Pro Ala Leu Pro His Gly Ser Lys His Arg Val Tyr Phe Asn Thr
            340                 345                 350

Pro Asn Gly Pro Leu Glu Arg Val Pro Ala Trp Ala Asn Phe Val Ile
        355                 360                 365

Pro Asp Ala Asp Gly Met Ala Leu Ala Val His Trp Glu Pro Pro Pro
    370                 375                 380

Glu Tyr Ala Tyr Lys Trp Lys His Lys Leu Pro Val Lys Pro Lys Ser
385                 390                 395                 400

Leu Arg Ile Tyr Glu Cys His Val Gly Ile Ser Gly Gln Glu Pro Lys
                405                 410                 415

Val Ser Ser Phe Asn Asp Phe Ile Ser Lys Val Leu Pro His Val Lys
            420                 425                 430

Glu Ala Gly Tyr Asn Ala Thr Gln Ile Ile Gly Val Val Glu His Lys
        435                 440                 445
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Asp Tyr Phe Thr Val Gly Tyr Arg Val Thr Asn Phe Tyr Ala Val Ser
 450                 455                 460

Ser Arg Tyr Gly Thr Pro Asp Asp Phe Lys Arg Leu Val Asp Glu Ala
465                 470                 475                 480

His Gly Leu Gly Leu Leu Val Phe Leu Glu Ile Val His Ser Tyr Ala
                485                 490                 495

Ala Ala Asp Glu Met Val Gly Leu Ser Leu Phe Asp Gly Ala Asn Asp
            500                 505                 510

Cys Tyr Phe His Thr Gly Lys Arg Gly His His Lys Phe Trp Gly Thr
        515                 520                 525

Arg Met Phe Lys Tyr Gly Asp Pro Asp Val Leu His Phe Leu Leu Ser
 530                 535                 540

Asn Leu Asn Trp Trp Val Glu Glu Tyr His Val Asp Gly Phe His Phe
545                 550                 555                 560

His Ser Leu Ser Ser Met Leu Tyr Thr His Asn Gly Phe Ala Ser Phe
                565                 570                 575

Thr Gly Asp Met Asp Glu Tyr Cys Asn Gln Tyr Val Asp Lys Glu Ala
            580                 585                 590

Leu Leu Tyr Leu Ile Leu Ala Asn Glu Val Leu His Ala Leu His Pro
        595                 600                 605

Asn Val Ile Thr Ile Ala Val Asp Ala Thr Leu Tyr Pro Gly Leu Cys
 610                 615                 620

Asp Pro Thr Ser Gln Gly Gly Leu Gly Phe Asp Tyr Phe Ala Asn Leu
625                 630                 635                 640

Ser Ala Ser Glu Met Trp Leu Ala Leu Leu Glu Asn Thr Pro Asp His
                645                 650                 655

Glu Trp Cys Met Ser Lys Ile Val Ser Thr Leu Val Gly Asp Arg Gln
            660                 665                 670

Asn Thr Asp Lys Met Leu Leu Tyr Ala Glu Asn His Asn Gln Ser Ile
        675                 680                 685

Ser Gly Gly Arg Ser Phe Ala Glu Ile Leu Ile Gly Asn Ser Leu Gly
 690                 695                 700

Lys Ser Ser Ile Ser Gln Glu Ser Leu Leu Arg Gly Cys Ser Leu His
705                 710                 715                 720
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Lys Met Ile Arg Leu Ile Thr Ser Thr Ile Gly Gly His Ala Tyr Leu
725 730 735

Asn Phe Met Gly Asn Glu Phe Gly His Pro Lys Arg Val Glu Phe Pro
740 745 750

Met Ser Ser Asn Asn Phe Ser Phe Ser Leu Ala Asn Arg Arg Trp Asp
755 760 765

Leu Leu Glu Asp Val Val His Tyr Gln Leu Phe Ser Phe Asp Lys Asp
770 775 780

Met Met Asp Leu Asp Lys Asn Gly Arg Ile Leu Ser Arg Gly Leu Ala
785 790 795 800

Asn Ile His His Val Asn Asp Thr Thr Met Val Ile Ser Tyr Leu Arg
805 810 815

Gly Pro Asn Leu Phe Val Phe Asn Phe His Pro Val Asn Ser Tyr Glu
820 825 830

Arg Tyr Ile Ile Gly Val Glu Glu Ala Gly Glu Tyr Gln Val Thr Leu
835 840 845

Asn Thr Asp Glu Asn Lys Tyr Gly Gly Arg Gly Leu Leu Gly His Asp
850 855 860

Gln Asn Thr Gln Arg Thr Ile Ser Arg Arg Ala Asp Gly Met Arg Phe
865 870 875 880

Cys Leu Glu Val Pro Leu Pro Ser Arg Ser Ala Gln Val Tyr Lys Leu
885 890 895

Thr Arg Ile Leu Arg Ala
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<213> Solanum tuberosum

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     1               5                  10                  15

gat tct cgt ctt agt ttt cta tct caa acc gga agc aga acc agt cgc      97
Asp Ser Arg Leu Ser Phe Leu Ser Gln Thr Gly Ser Arg Thr Ser Arg
                 20                  25                  30

cag ctt aaa ttt gtt cgc agc cgc cgg gct cga gtt tcg agg tgt aga     145
Gln Leu Lys Phe Val Arg Ser Arg Arg Ala Arg Val Ser Arg Cys Arg
             35                  40                  45

tgc tca gca acg gag caa ccg cca ccg caa cga cgg aag caa cga ccg     193
Cys Ser Ala Thr Glu Gln Pro Pro Pro Gln Arg Arg Lys Gln Arg Pro
         50                  55                  60

gag aag tac aaa cag tcg gag gaa ggg aaa gga atc gat cct gtt gga     241
Glu Lys Tyr Lys Gln Ser Glu Glu Gly Lys Gly Ile Asp Pro Val Gly
     65                  70                  75

ttt ctc agc aaa tac ggc att act cat aaa gcg ttt gct caa ttt ctt     289
Phe Leu Ser Lys Tyr Gly Ile Thr His Lys Ala Phe Ala Gln Phe Leu
80                  85                  90                  95

cgt gaa aga tat aaa tca ttg aag gac ttg aag gat gaa ata ttg act     337
Arg Glu Arg Tyr Lys Ser Leu Lys Asp Leu Lys Asp Glu Ile Leu Thr
                100                 105                 110

cgt cat ttc agt ctc aag gag atg tct act ggg tat gaa tta atg ggt     385
Arg His Phe Ser Leu Lys Glu Met Ser Thr Gly Tyr Glu Leu Met Gly
            115                 120                 125

atg cat cgc aac ata caa cat cga gtg gat ttc ttg gaa tgg gct cca     433
Met His Arg Asn Ile Gln His Arg Val Asp Phe Leu Glu Trp Ala Pro
        130                 135                 140

ggt gct cgc tac tgt gct ctg att ggt gac ttc aat ggg tgg tca aca     481
Gly Ala Arg Tyr Cys Ala Leu Ile Gly Asp Phe Asn Gly Trp Ser Thr
    145                 150                 155

act ggt aac tgt gcc aga gag ggt cat ttt ggt cat gac gat tat ggg     529
Thr Gly Asn Cys Ala Arg Glu Gly His Phe Gly His Asp Asp Tyr Gly
160                 165                 170                 175

tat tgg ttt att att ctt gaa gat aaa tta cgt gaa gga gaa gaa cct     577
Tyr Trp Phe Ile Ile Leu Glu Asp Lys Leu Arg Glu Gly Glu Glu Pro
                180                 185                 190

gat aaa ttg tat ttt caa cag tac aat tat gcg gag gac tat ggt aaa     625
Asp Lys Leu Tyr Phe Gln Gln Tyr Asn Tyr Ala Glu Asp Tyr Gly Lys
            195                 200                 205

ggt gac acg ggt att acc gtc gag gaa atc ttt aaa aaa gca aat gat     673
Gly Asp Thr Gly Ile Thr Val Glu Glu Ile Phe Lys Lys Ala Asn Asp
        210                 215                 220

gag tat tgg gaa cct gga gaa gat cgc ttc att aaa tca cgt tat gag     721
Glu Tyr Trp Glu Pro Gly Glu Asp Arg Phe Ile Lys Ser Arg Tyr Glu
    225                 230                 235

gtg gca gca aag tta tat gag gaa atg ttc gga cca aat gga cct caa     769
Val Ala Ala Lys Leu Tyr Glu Glu Met Phe Gly Pro Asn Gly Pro Gln
240                 245                 250                 255

aca gaa gag gaa cta gaa gca atg cct gat gca gct aca cga tac aaa     817
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Thr Glu Glu Glu Leu Glu Ala Met Pro Asp Ala Ala Thr Arg Tyr Lys
                260                 265                 270

act tgg aaa gag caa caa aaa gag gat ccg gca agc aat ttg cca tcg      865
Thr Trp Lys Glu Gln Gln Lys Glu Asp Pro Ala Ser Asn Leu Pro Ser
            275                 280                 285

tat gat gtg gta gat agt gga aaa gaa tat gat att tac aat att ata      913
Tyr Asp Val Val Asp Ser Gly Lys Glu Tyr Asp Ile Tyr Asn Ile Ile
        290                 295                 300

ggt gat cct gaa tcg ttt aag aaa ttt cgt atg aaa cag cct cct att      961
Gly Asp Pro Glu Ser Phe Lys Lys Phe Arg Met Lys Gln Pro Pro Ile
    305                 310                 315

gct tac tgg tta gaa act aaa aag gga agg aaa ggc tgg tta cag aaa     1009
Ala Tyr Trp Leu Glu Thr Lys Lys Gly Arg Lys Gly Trp Leu Gln Lys
320                 325                 330                 335

tat atg cct gct tta cct cat gga agc aaa tac agg gtg tat ttt aac     1057
Tyr Met Pro Ala Leu Pro His Gly Ser Lys Tyr Arg Val Tyr Phe Asn
                340                 345                 350

aca cca aat ggg cct ctt gaa cga gtt cct gcg tgg gcc aat ttt gtc     1105
Thr Pro Asn Gly Pro Leu Glu Arg Val Pro Ala Trp Ala Asn Phe Val
            355                 360                 365

att cca gat gca ggc ggg atg gca tta gca gtc cat tgg gaa cca cct     1153
Ile Pro Asp Ala Gly Gly Met Ala Leu Ala Val His Trp Glu Pro Pro
        370                 375                 380

cct gaa tat gct tat aaa tgg aaa cac aag cta cca gtc aag cct aag     1201
Pro Glu Tyr Ala Tyr Lys Trp Lys His Lys Leu Pro Val Lys Pro Lys
    385                 390                 395

tcc ttg cgc ata tat gaa tgt cat gtt ggc atc tct ggc cag gaa cca     1249
Ser Leu Arg Ile Tyr Glu Cys His Val Gly Ile Ser Gly Gln Glu Pro
400                 405                 410                 415

aaa gtt tca tct ttc aat gat ttt att agc aag gtc ctt ccg cat gta     1297
Lys Val Ser Ser Phe Asn Asp Phe Ile Ser Lys Val Leu Pro His Val
                420                 425                 430

aaa gaa gct gga tac aat gca ata caa att att gga gtt gtt gag cac     1345
Lys Glu Ala Gly Tyr Asn Ala Ile Gln Ile Ile Gly Val Val Glu His
            435                 440                 445

aag gat tat ttc act gtt gga tat aga gtg acc aat ttt tat gct gtt     1393
Lys Asp Tyr Phe Thr Val Gly Tyr Arg Val Thr Asn Phe Tyr Ala Val
        450                 455                 460

agt agc cgt tat ggc aca ccg gat gac ttc aag cgc ttg gtt gat gaa     1441
Ser Ser Arg Tyr Gly Thr Pro Asp Asp Phe Lys Arg Leu Val Asp Glu
    465                 470                 475

gca cat ggg ctt gga ctg ctt gtc ttt ttg gag att gtg cac tct tat     1489
Ala His Gly Leu Gly Leu Leu Val Phe Leu Glu Ile Val His Ser Tyr
480                 485                 490                 495

gca gca gca gat gaa atg gtt ggg tta tct ctt ttt gat gga gca aat     1537
Ala Ala Ala Asp Glu Met Val Gly Leu Ser Leu Phe Asp Gly Ala Asn
                500                 505                 510

gat tgc tat ttc cac act ggt aaa cgt gga cac cac aaa ttc tgg ggc     1585
Asp Cys Tyr Phe His Thr Gly Lys Arg Gly His His Lys Phe Trp Gly
            515                 520                 525

aca cgg atg ttc aaa tat gga gat ctt gat gtt ctg cac ttt ctt ctt     1633
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Ser Asn Leu Asn Trp Trp Val Glu Glu Tyr His Val Asp Gly Phe His
    545                 550                 555

ttt cat tcg ctc tcg tcc atg ttg tat acg cat aat gga ttt gct tca  1729
Phe His Ser Leu Ser Ser Met Leu Tyr Thr His Asn Gly Phe Ala Ser
560                 565                 570                 575

ttt act ggt gac atg gat gaa tac tgt aac caa tat gtt gac aag gag  1777
Phe Thr Gly Asp Met Asp Glu Tyr Cys Asn Gln Tyr Val Asp Lys Glu
                580                 585                 590

gcc tta ttg tac ctc ata tta gca aat gaa gta tta cat gct ctt cat  1825
Ala Leu Leu Tyr Leu Ile Leu Ala Asn Glu Val Leu His Ala Leu His
            595                 600                 605

cct aat gtg atc acg att gct gag gat gca act ctg tat cct gga ctc  1873
Pro Asn Val Ile Thr Ile Ala Glu Asp Ala Thr Leu Tyr Pro Gly Leu
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tgc gat cca aca tct caa ggt gga ctg ggc ttt gat tat ttt gcc aat  1921
Cys Asp Pro Thr Ser Gln Gly Gly Leu Gly Phe Asp Tyr Phe Ala Asn
    625                 630                 635

ctt tct gcc tca gag atg tgg ctt gca tta ctt gaa aat act cct gat  1969
Leu Ser Ala Ser Glu Met Trp Leu Ala Leu Leu Glu Asn Thr Pro Asp
640                 645                 650                 655

cat gaa tgg tgc atg agt aag att gtt agc aca tta gtg ggc gat aga  2017
His Glu Trp Cys Met Ser Lys Ile Val Ser Thr Leu Val Gly Asp Arg
                660                 665                 670

caa aat act gat aaa atg ctt ttg tat gca gaa aat cac aac cag tcc  2065
Gln Asn Thr Asp Lys Met Leu Leu Tyr Ala Glu Asn His Asn Gln Ser
            675                 680                 685

att tct gga ggt cgt tcc ttc gca gaa ata ctg att ggt aac tcc ttg  2113
Ile Ser Gly Gly Arg Ser Phe Ala Glu Ile Leu Ile Gly Asn Ser Leu
        690                 695                 700

ggg aaa tcc tcc ata tca caa gag tca tta ctt aga ggc tgc tcg tta  2161
Gly Lys Ser Ser Ile Ser Gln Glu Ser Leu Leu Arg Gly Cys Ser Leu
    705                 710                 715

cac aag atg atc aga tta att aca tct aca att ggt ggt cat gca tac  2209
His Lys Met Ile Arg Leu Ile Thr Ser Thr Ile Gly Gly His Ala Tyr
720                 725                 730                 735

ctc aac ttc atg ggc aat gaa ttt ggt cac cca aag aga gta gag ttt  2257
Leu Asn Phe Met Gly Asn Glu Phe Gly His Pro Lys Arg Val Glu Phe
                740                 745                 750

cca atg tca agc aac aat ttc tcc ttt tca ctg gct aac cgt cgc tgg  2305
Pro Met Ser Ser Asn Asn Phe Ser Phe Ser Leu Ala Asn Arg Arg Trp
            755                 760                 765

gat cta ttg gaa gat gtt gta cat tat caa ttg ttc tca ttt gat aag  2353
Asp Leu Leu Glu Asp Val Val His Tyr Gln Leu Phe Ser Phe Asp Lys
        770                 775                 780

ggt atg atg gac ttg gat aaa aat ggg aga att ttg tcc aga ggt ctt  2401
Gly Met Met Asp Leu Asp Lys Asn Gly Arg Ile Leu Ser Arg Gly Leu
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gcc aac att cac cat gtc aat gat act acc atg gtg att tct tac ttg  2449
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Ala Asn Ile His His Val Asn Asp Thr Thr Met Val Ile Ser Tyr Leu
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aga ggt ccc aat ctc ttt gtg ttc aac ttt cat cct gtc aat tca tat      2497
Arg Gly Pro Asn Leu Phe Val Phe Asn Phe His Pro Val Asn Ser Tyr
                820                 825                 830

gaa aga tac att ata ggt gtg gaa gaa gct gga gag tat caa gtc aca      2545
Glu Arg Tyr Ile Ile Gly Val Glu Glu Ala Gly Glu Tyr Gln Val Thr
            835                 840                 845

tta aat aca gat gaa aac aag tat ggt ggt aga gga cta ctt ggc cat      2593
Leu Asn Thr Asp Glu Asn Lys Tyr Gly Gly Arg Gly Leu Leu Gly His
        850                 855                 860

gat cag aat att caa aga acc att agt aga aga gct gat gga atg aga      2641
Asp Gln Asn Ile Gln Arg Thr Ile Ser Arg Arg Ala Asp Gly Met Arg
    865                 870                 875

ttt tgc ttg gaa gtg cct ctg cca agt aga agt gct cag gtc tac aag      2689
Phe Cys Leu Glu Val Pro Leu Pro Ser Arg Ser Ala Gln Val Tyr Lys
880                 885                 890                 895

ttg acc cga att cta aga gca tgatcactct agtaatcaaa gtgcctcata        2740
Leu Thr Arg Ile Leu Arg Ala
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gccttcagca tccggatagt cgcttctcct gtgcaatgag ggcatggacg aattttttt   2920

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Leu Lys Phe Val Arg Ser Arg Arg Ala Arg Val Ser Arg Cys Arg Cys
        35                  40                  45

Ser Ala Thr Glu Gln Pro Pro Pro Gln Arg Arg Lys Gln Arg Pro Glu
    50                  55                  60
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Lys Tyr Lys Gln Ser Glu Glu Gly Lys Gly Ile Asp Pro Val Gly Phe
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Leu Ser Lys Tyr Gly Ile Thr His Lys Ala Phe Ala Gln Phe Leu Arg
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Glu Arg Tyr Lys Ser Leu Lys Asp Leu Lys Asp Glu Ile Leu Thr Arg
            100                 105                 110

His Phe Ser Leu Lys Glu Met Ser Thr Gly Tyr Glu Leu Met Gly Met
        115                 120                 125

His Arg Asn Ile Gln His Arg Val Asp Phe Leu Glu Trp Ala Pro Gly
    130                 135                 140

Ala Arg Tyr Cys Ala Leu Ile Gly Asp Phe Asn Gly Trp Ser Thr Thr
145                 150                 155                 160

Gly Asn Cys Ala Arg Glu Gly His Phe Gly His Asp Asp Tyr Gly Tyr
                165                 170                 175

Trp Phe Ile Ile Leu Glu Asp Lys Leu Arg Glu Gly Glu Glu Pro Asp
            180                 185                 190

Lys Leu Tyr Phe Gln Gln Tyr Asn Tyr Ala Glu Asp Tyr Gly Lys Gly
        195                 200                 205

Asp Thr Gly Ile Thr Val Glu Glu Ile Phe Lys Lys Ala Asn Asp Glu
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Tyr Trp Glu Pro Gly Glu Asp Arg Phe Ile Lys Ser Arg Tyr Glu Val
225                 230                 235                 240

Ala Ala Lys Leu Tyr Glu Glu Met Phe Gly Pro Asn Gly Pro Gln Thr
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Glu Glu Glu Leu Glu Ala Met Pro Asp Ala Ala Thr Arg Tyr Lys Thr
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Trp Lys Glu Gln Gln Lys Glu Asp Pro Ala Ser Asn Leu Pro Ser Tyr
        275                 280                 285

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Tyr Trp Leu Glu Thr Lys Lys Gly Arg Lys Gly Trp Leu Gln Lys Tyr
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Pro Asp Ala Gly Gly Met Ala Leu Ala Val His Trp Glu Pro Pro Pro
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Glu Tyr Ala Tyr Lys Trp Lys His Lys Leu Pro Val Lys Pro Lys Ser
385 390 395 400

Leu Arg Ile Tyr Glu Cys His Val Gly Ile Ser Gly Gln Glu Pro Lys
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Glu Ala Gly Tyr Asn Ala Ile Gln Ile Ile Gly Val Val Glu His Lys
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Ser Arg Tyr Gly Thr Pro Asp Asp Phe Lys Arg Leu Val Asp Glu Ala
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His Gly Leu Gly Leu Leu Val Phe Leu Glu Ile Val His Ser Tyr Ala
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Ala Ala Asp Glu Met Val Gly Leu Ser Leu Phe Asp Gly Ala Asn Asp
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Cys Tyr Phe His Thr Gly Lys Arg Gly His His Lys Phe Trp Gly Thr
515 520 525

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625 630 635 640

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660 665 670

Asn Thr Asp Lys Met Leu Leu Tyr Ala Glu Asn His Asn Gln Ser Ile
675 680 685

Ser Gly Gly Arg Ser Phe Ala Glu Ile Leu Ile Gly Asn Ser Leu Gly
690 695 700

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Lys Met Ile Arg Leu Ile Thr Ser Thr Ile Gly Gly His Ala Tyr Leu
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740 745 750

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Leu Leu Glu Asp Val Val His Tyr Gln Leu Phe Ser Phe Asp Lys Gly
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785 790 795 800

Asn Ile His His Val Asn Asp Thr Thr Met Val Ile Ser Tyr Leu Arg
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Gly Pro Asn Leu Phe Val Phe Asn Phe His Pro Val Asn Ser Tyr Glu
820 825 830

Arg Tyr Ile Ile Gly Val Glu Glu Ala Gly Glu Tyr Gln Val Thr Leu
835 840 845

Asn Thr Asp Glu Asn Lys Tyr Gly Gly Arg Gly Leu Leu Gly His Asp
850 855 860

Gln Asn Ile Gln Arg Thr Ile Ser Arg Arg Ala Asp Gly Met Arg Phe
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Claims

1. A potato starch which exhibits an amylose content, as measured by the method of Hovenkamp-Hermelink et al. (1988, Potato Research 31, 241-246), of less than 10% by weight, a phosphate content in the C6 position, as measured by method b) "Determining the phosphate content in the C6 position" described in section "General methods", 2. Starch analysis, of between 35 and 100 nmol of phosphate per milligram of starch (dry weight) and a content of side chains, as measured by method f) "Using gel permeation chromatography to analyze the side chain distribution in total starch" described in section "General methods", 2. Starch analysis, having a DP of from 12 to 19 which is increased to 125%-200% as compared with that in potato starch from corresponding wild-type potato plants.

2. The potato starch as claimed in claim 1, which exhibits a phosphate content in the C6 position of between 40 and 85 nmol of phosphate per milligram of starch (dry weight).

3. The potato starch as claimed in claim 1, which exhibits a phosphate content in the C6 position of between 45 and 70 nmol of phosphate per milligram of starch (dry weight).

4. The potato starch as claimed in claim 1, which exhibits a phosphate content in the C6 position of between 50 and 65 nmol of phosphate per milligram of starch (dry weight).

5. The potato starch as claimed in one of claims 1 to 4, which exhibits an amylose content, as measured by the method of Hovenkamp-Hermelink et al. (1988, Potato Research 31, 241-246), of less than 5% by weight

6. The potato starch as claimed in one of claims 1 to 4, which exhibits an amylose content, as measured by the method of Hovenkamp-Hermelink et al. (1988, Potato Research 31,241-246), of less than 3% by weight.

7. The potato starch as claimed in one of claims 1 to 6, wherein the proportion of side chains having a DP of from 12 to 19 is increased to 130%-180% as compared with potato starch from corresponding wild-type potato plants.

8. The potato starch as claimed in one of claims 1 to 7, wherein the proportion of side chains having a DP of from 63 to 123 is reduced as compared with potato starch from corresponding wild-type potato plants.

9. The potato starch as claimed in claim 8, wherein the proportion of side chains having a DP of from 63 to 123 is reduced to 50%-95% as compared with potato starch from corresponding wild-type potato plants.

10. A derivatized potato starch which contains potato starch as claimed in one of claims 1 to 9.

Patentansprüche

1. Kartoffelstärke, die einen Amylosegehalt gemessen nach der Methode von Hovenkamp-Hermelink et al. (1988, Potato Research 31, 241-246) von weniger als 10 Gew.-%, einen Phosphatgehalt in C6-Position gemessen nach der im Abschnitt "Allgemeine Methoden", 2. Stärkeanalytik, beschriebenen Methode b) "Bestimmung des Phosphatgehaltes in C6-Position" zwischen 35 und 100 nmol Phosphat pro Milligramm Stärke (Trockengewicht) und einen auf 125%-200% erhöhten Gehalt an Seitenketten gemessen nach der im Abschnitt "Allgemeine Methoden", 2. Stärkeanalytik, beschriebenen Methode f) "Analyse der Seitenkettenverteilung von Gesamtstärke mittels Gelpermeationschromatographie" mit einem DP von 12 bis 19 im Vergleich zu Kartoffelstärke aus entsprechenden Kartoffel-Wildtyp-Pflanzen aufweist.

2. Kartoffelstärke nach Anspruch 1, die einen Phosphatgehalt in C6-Position zwischen 40 und 85 nmol Phosphat pro Milligramm Stärke (Trockengewicht) aufweist.

3. Kartoffelstärke nach Anspruch 1, die einen Phosphatgehalt in C6-Position zwischen 45 und 70 nmol Phosphat pro Milligramm Stärke (Trockengewicht) aufweist.

4. Kartoffelstärke nach Anspruch 1, die einen Phosphatgehalt in C6-Position zwischen 50 und 65 nmol Phosphat pro Milligramm Stärke (Trockengewicht) aufweist.

5. Kartoffelstärke nach einem der Ansprüche 1 bis 4, die einen Amylosegehalt gemessen nach der Methode von Hovenkamp-Hermelink et al. (1988, Potato Research 31, 241-246) von weniger als 5 Gew.-% aufweist.

6. Kartoffelstärke nach einem der Ansprüche 1 bis 4, die einen Amylosegehalt gemessen nach der Methode von Hovenkamp-Hermelink et al. (1988, Potato Research 31, 241-246) von weniger als 3 Gew.-% aufweist.

7. Kartoffelstärke nach einem der Ansprüche 1 bis 6, wobei der Anteil der Seitenketten mit einem DP von 12 bis 19 auf 130%-180% erhöht ist im Vergleich zu Kartoffelstärke aus entsprechenden Kartoffel-Wildtyp-Pflanzen.

8. Kartoffelstärke nach einem der Ansprüche 1 bis 7, wobei der Anteil der Seitenketten mit einem DP von 63 bis 123 verringert ist im Vergleich zu Kartoffelstärke aus entsprechenden Kartoffel-Wildtyp-Pflanzen.

9. Kartoffelstärke nach Anspruch 8, wobei der Anteil der Seitenketten mit einem DP von 63 bis 123 auf 50%-95% verringert ist im Vergleich zu Kartoffelstärke aus entsprechenden Kartoffel-Wildtyp-Pflanzen.

10. Derivatisierte Kartoffelstärke enthaltend Kartoffelstärke nach einem der Ansprüche 1 bis 9.

Revendications

1. Amidon de pomme de terre qui présente une teneur en amylose, mesuré par le procédé de Hovenkamp-Hermellnk et al. (1988, Potato Research 31, 241-246), inférieure à 10 % en poids, une teneur en phosphate à la position C6, mesurée par le procédé b) « Determining the phosphate content in the C6 position » décrite dans la section « General methods », 2. Starch analysis, comprise entre 35 et 100 nmol de phosphate par milligramme d'amidon (poids sec) et une teneur en chaînes latérales, mesurée par le procédé f) « Using gel permeation chromatography to analyze the side chain distribution in total starch » décrit dans la section « General methods », 2. Starch analysis, ayant un DP de 12 à 19 qui est augmenté de 125 à 200 % par rapport à celui d'amidon de pomme de terre de plantes de pomme de terre de type sauvage correspondantes.

2. Amidon de pomme de terre selon la revendication 1, qui présente une teneur en phosphate à la position C6 comprise entre 40 et 85 nmol de phosphate par milligramme d'amidon (poids sec).

3. Amidon de pomme de terre selon la revendication 1, qui présente une teneur en phosphate à la position C6 comprise entre 45 et 70 nmol de phosphate par milligramme d'amidon (poids sec).

4. Amidon de pomme de terre selon la revendication 1, qui présente une teneur en phosphate à la position C6 comprise entre 50 et 65 nmol de phosphate par milligramme d'amidon (poids sec).

5. Amidon de pomme de terre selon l'une des revendications 1 à 4, qui présente une teneur en amylose, mesurée par le procédé de Hovenkamp-Hermellnk et al. (1988, Potato Research 31, 241-246), inférieure à 5 % en poids.

6. Amidon de pomme de terre selon une des revendications 1 à 4, qui présente une teneur en amylose, mesurée par le procédé de Hovenkamp-Hermellnk et al. (1988, Potato Research 31, 241-246), inférieure à 3 % en poids.

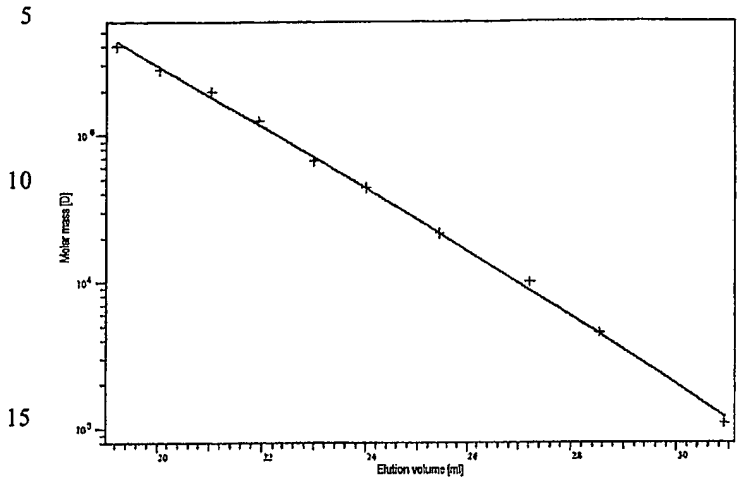
7. Amidon de pomme de terre selon une des revendications 1 à 6, dans lequel la proportion de chaînes latérales ayant un DP de 12 à 19 est augmentée à 130 % à 180 % par rapport à l'amidon de pomme de terre de plantes de pomme de terre de type sauvage correspondantes.

8. Amidon de pomme de terre selon une des revendications 1 à 7, dans lequel la proportion de chaînes latérales ayant un DP de 63 à 123 est réduite par rapport à l'amidon de pomme de terre de plantes de pomme de terre de type sauvage correspondantes.

9. Amidon de pomme de terre selon la revendication 8, dans lequel la proportion de chaînes latérales ayant un DP de 63 à 123 est réduite à 50 % à 95 % par rapport à l'amidon de pomme de terre de plantes de pomme de terre de type sauvage correspondantes.

10. Amidon de pomme de terre dérivé qui contient de l'amidon de pomme de terre selon les revendications 1 à 9.

Figure 1



Fit quality:
Fit type: PSS Poly 3
R : 0.999274

Mark-Houwink coefficients:
a = 0.000 K = 1.0000

Elution volume [ml]	Molar mass [D]	Sample name	Slope	Deviation [%]
19.22	401300	Dextran T670P	-0.21	8.02
20.05	276500	Dextran T410P	-0.21	4.83
21.03	196300	Dextran T270P	-0.21	-8.08
21.93	123600	Dextran T150P	-0.21	-5.29
22.98	66700	Dextran T80	-0.21	5.87
24.00	43500	Dextran T50	-0.21	-0.75
25.43	21400	Dextran T25	-0.21	0.11
27.22	9890	Dextran T12	-0.22	-11.73
28.55	4440	Dextran T5	-0.23	-1.62
30.92	1080	Dextran T1	-0.25	11.05

REFERENCES CITED IN THE DESCRIPTION

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